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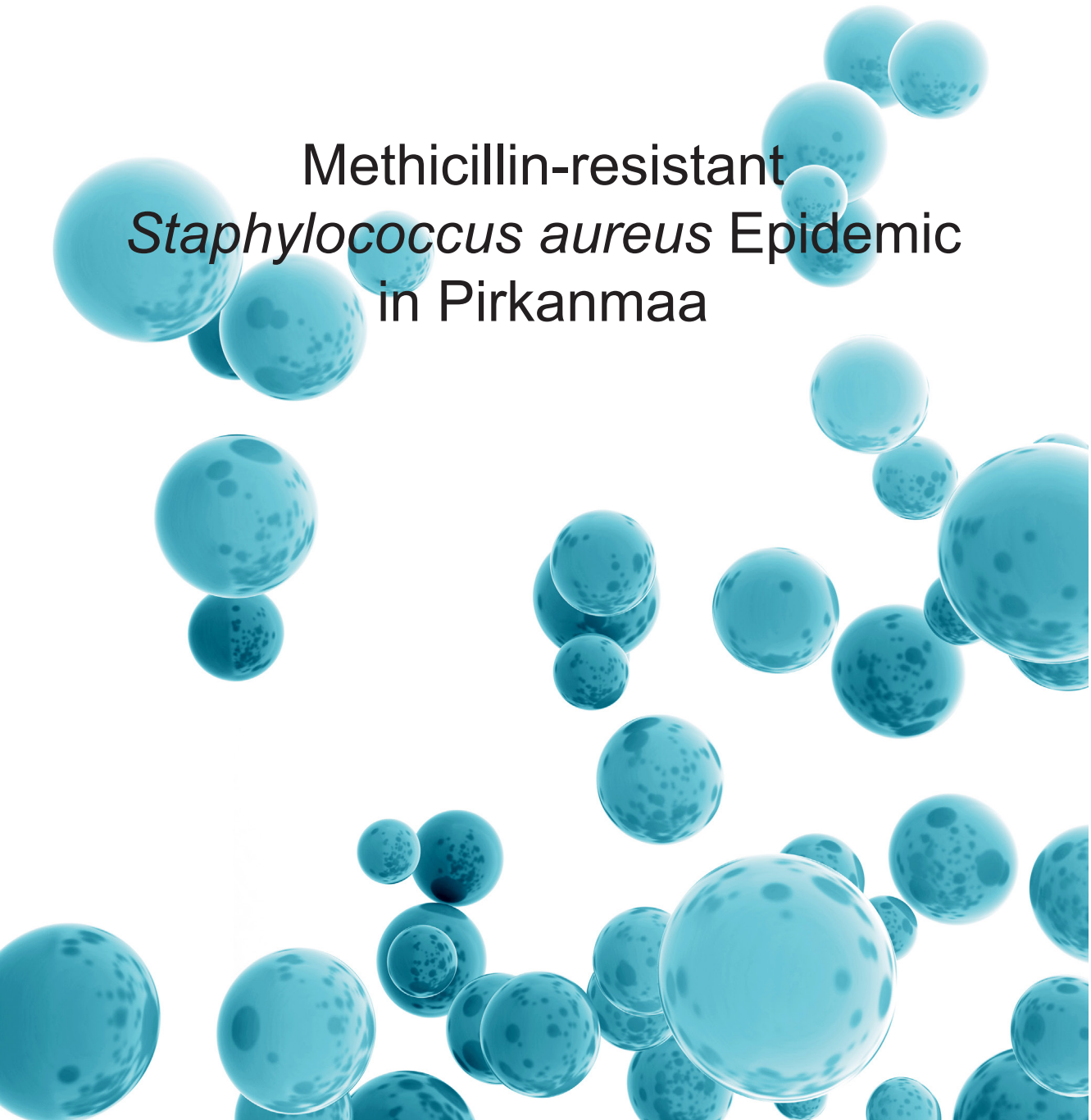
ELINA JOKINEN

Methicillin-resistant *Staphylococcus aureus* Epidemic in Pirkanmaa



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ABSTRACT

Staphylococcus aureus (*S.aureus*) causes a significant burden to the population. Infections are expensive to treat and associated with long hospitalizations. Regardless of modern treatment, serious *S. aureus* infections are still associated with significant mortality. The emergence of *S. aureus* strains that are resistant to commonly used beta-lactam antibiotics (methicillin-resistant staphylococcus aureus, MRSA) have made the treatment of infections even more challenging. *S. aureus* is a common cause of both community-acquired (CA) and health care associated (HA) infections.

A MRSA epidemic broke out in Pirkanmaa county in 2001. Within the epidemic, a large number MRSA transmission and infections have been diagnosed. To combat the epidemic, several infection control measures were established and more resources were allocated for prevention work. The aim of this study was to assess the impact of improved hospital hygiene on MRSA transmissions and bacteremias. Another aim was to study whether improved hospital hygiene affected the occurrence of bacteremias caused by methicillin susceptible strains and to study the proportions of penicillin-susceptible (PSSA) and penicillin-resistant (PRSA) strains in *S.aureus* bacteremia. The clinical characteristics of MRSA, PRSA and PSSA bacteremias were compared.

S. aureus strains can be distinguished using several typing methods. Strain distinction enables identification of transmission chains that are related to the epidemic and of sporadic cases that are not related to the epidemic. This data helps targeting and resourcing infection control measures. One method for strain distinction is spa typing. It is based on single-locus DNA sequencing of polymorphic region of the *spa* gene that codes the *S. aureus*-specific staphylococcal protein A. Spa types can be further assigned to clonal complexes (spaCC) based on their relatedness. Protein A is also one of the several virulence factors of *S. aureus* and thus spa type may be related to the severity of *S. aureus* bacteremia. Spa type distribution in HA-MRSA transmissions, in symptomless MRSA carriers and in MRSA and MSSA bacteremias was analysed. Also the association of spa type with the clinical characteristics of *S. aureus* bacteremia was studied.

The study material consists of data from infection surveillance registers. Data was obtained from the MRSA register run by the Infectious Diseases Unit in Tampere University Hospital, the Commercial Surveillance System of Antibiotics and Infections (SAI), National Institute for Health and Welfare's (THL) National Infectious Diseases Register, Finnish Hospital Infection Program (SIRO) and the National Hospital Discharge Database (HILMO) and hospital records.

There were 4118 MRSA transmission between 2001-2014 and 3527 (85.6%) of them were HA. Transmissions were particularly common in long-term facilities. The setting of a transmission was defined using an algorithm that takes into account previous treatment periods in health care or long-term care units. During the first two years of the epidemic 71.2% of the HA-MRSA findings were recovered from clinical samples, while in 2014 only 9.4% were found in such samples. Of cases, 78.7% were caused by a single strain, t067. The proportion of the epidemic strains decreased from 98.0% to 45.6% in during the study period. The number of annual MRSA transmissions increased until the year 2011 but almost halved since then. As a part of infection control, universal MRSA screening of patients admitted to hospitals was implemented in 2011. This has led to increased detection of sporadic MRSA cases. As a surrogate marker of improved hand hygiene, the use of alcohol hand rub more than doubled during the study period.

Between years 2005 and 2015 the incidence of *S. aureus* bacteremia increased from 21.6 to 35.8 per 100 000 population and the increasing trend appears to be similar in both HA and CA cases. Of cases, 54.7% were HA. The incidence of MRSA bacteremias increased until the year 2011 but decreased thereafter. At its height, the incidence of MRSA bacteremia was 10-fold (4.5/100 000) compared to other parts of the country but decreased since then to the average level of the country (0.6/100 000). The decline of MRSA bacteremias occurred simultaneously with the decline of MRSA transmissions. Of bacteremias, 82.6% were caused by the epidemic strain, t067. In MSSA bacteremias, spa type distribution was more diverse and no dominating strain could be identified. The majority of MSSA bacteremias were caused by PRSA, but the proportion of PSSA increased significantly from 23.9% to 43.1%.

In this study the risk of having MRSA bacteremia compared to MSSA bacteremia was greater in patients who were smokers, on glucocorticoid therapy, had previous surgery or whose bacteremia was HA. MRSA bacteremia was associated with greater mortality (OR 2.0, 95% CI 1.20-3.34) and the difference remained significant even after adjusting with age, comorbidities and the focus of

infection. Of focus, pneumonia was associated with higher mortality (OR 2.51, 95% CI 1.42-4.44) in *S. aureus* bacteremia but skin and soft tissue infections and osteomyelitis and spondylodiscitis with lower mortality (OR 0.42, 95% CI 0.24-0.74 and 0.32, 95% CI 0.11-0.90, respectively). Previous glucocorticoid treatment, liver cirrhosis, older age, cardiac disease and unknown focus of infection were associated with higher mortality. Appropriate empiric therapy did not have an impact on the outcome in this study.

Case fatality decreased from year 2005 (21.8%) to year 2015 (17.1%). The highest mortality was associated with spa types t002 and t067 (27.6% and 26.9%, respectively). Of clonal complexes, spaCC 067 was associated with the highest mortality (25.6%). The results of this study also suggest that spaCC 008 is more often associated with endocarditis, spaCC 012 with foreign body infections and spaCC 2133 with skin and soft tissue infections than other spaCCs.

In conclusion, during the recent years both HA-MRSA transmissions and MRSA bacteremias decreased significantly in Pirkanmaa county and this is probably mainly due to improved hospital hygiene. As the rise and fall of the epidemic is closely related with changes in the occurrence of t067, it is possible that also natural strain dynamics play a role. Regardless of improved hospital hygiene, bacteremias caused by susceptible *S. aureus* strains have increased. This is at least partly due to ageing of the population and increase of invasive medical procedures but also due to better diagnostics. The reason for the proportional increase of PSSA is unclear. No single epidemic strain was found amongst PSSA. As a novel observation, smoking and glucocorticoid therapy were found to be risk factors for MRSA bacteremia.

Improved survival of *S. aureus* bacteremia patients suggests that the quality of care may have improved. It is also possible, that cases of lesser severity were more frequently detected with higher blood screening rates. As noted earlier, MRSA bacteremia is associated with higher mortality than MSSA. There was no difference in mortality between PRSA and PSSA although there was a trend suggesting that mortality in PRSA bacteremia might be slightly higher. Unknown focus was associated with higher mortality, which emphasizes the importance of proper investigations to identify infective focus. The highest mortality was associated with spa types t002 and t067 and to spaCC 067. Spa t067 strains were almost exclusively methicillin resistant, which partly explains its association to the highest mortality. Moreover, t067 was very common in long-term facilities where dwellers probably are older and have underlying conditions that may impair their survival. Interestingly, both t067 and t002 belong to the same spaCC, which may indicate

that these strains share a common virulence factors associated with more severe disease.

The strain type distribution in MSSA bacteremias is very heterogenic. In MRSA bacteremias t067 was even more common than in MRSA screening samples suggesting that this strain readily causes invasive diseases. Wide distribution of t067 in Pirkanmaa and in Spain indicates that it also has properties that facilitate its spread. On the contrary, t1012 was very common in screening samples but has not caused any bacteremias in Pirkanmaa. This supports the perception that *S. aureus* strains differ in their ability to spread and cause infections. In this study, spaCC 008, 067 and 012 were related to certain focus of infection and severe forms of *S. aureus* disease.

TIIVISTELMÄ

Staphyloccous aureus (S. aureus) aiheuttaa merkittävän tautitaakan väestölle. Infektioiden hoito aiheuttaa pitkiä ja kalliita sairaalahoitojaksoja, ja vakaviin infektioihin liittyy nykyaikaisesta hoidosta huolimatta edelleen merkittävä kuolleisuus. Stafylokokki-infektioiden hoitoa vaikeuttaa yleisesti käytetylle beetalaktaamiantibiooteille vastustuskykyisten kantojen ilmaantuminen, erityisesti metisilliinille resistentit bakteerikannat (MRSA). *S. aureus* on tavallinen sekä avohoitoperäisten että hoitoon liittyvien infektioiden aiheuttaja.

Pirkanmaan sairaanhoitopiirissä alkoi laaja MRSA-epidemia vuonna 2001. Epidemiaan liittyen on todettu runsaasti uusia MRSA-tartuntoja ja MRSA:n aiheuttamia infektioita. Epidemian hillitsemiksi hoitoon liittyvien infektioiden torjuntaa on tehostettu monin tavoin ja MRSA-torjuntatyöhön on saatu resursseja. Tämän tutkimuksen tavoitteena oli selvittää, miten hoitoon liittyvien infektioiden torjunnan tehostaminen on vaikuttanut MRSA-tartuntojen ja bakteremioiden määrään. Tavoitteena oli myös tutkia, onko tehostunut hoitoon liittyvien infektioiden torjunta vaikuttanut antibiootille herkkien (MSSA) kantojen aiheuttamien bakteremioiden määrään. Lisäksi haluttiin tutkia MSSA kantojen alatyyppien, eli penisilliinille herkkien (PSSA) ja vastustuskykyisten (PRSA) kantojen osuutta *S. aureus*-bakteremioissa. MRSA:n, PRSA:n ja PSSA:n aiheuttamia bakteremioita vertailtiin keskenään.

S. aureus kannat voidaan erottaa toisistaan useilla eri menetelmillä. Kantayypityksen avulla voidaan tunnistaa epidemiaan liittyviä tartuntaketjuja ja toisaalta erottaa sporadiset, epidemiaan liittymättömät tapaukset. Tieto auttaa kohdistamaan ja resursoimaan epidemioiden torjuntatoimia. Spa-tyypitysmenetelmän kohteena on *spa*-geeni, joka koodaa bakteerin pinnalla sijaitsevaa proteiini A:ta. *Spa*-geenissä on vaihteleva määrä toistojaksoja, joiden sekvenssin ja lukumäärän perusteella määräytyy spa-tyyppi. Spa-tyypit voidaan edelleen ryhmitellä klonaalisiin komplekseihin (spaCC) kantojen sukulaisuuden perusteella. Proteiini A on myös yksi bakteerin monista virulenssitekijöistä, joten spa-tyypillä saattaa olla vaikutusta *S. aureuksen* aiheuttaman taudin vaikeusasteeseen. Tässä tutkimuksessa selvitettiin spa-tyyppien jakaumaa sairaalaperäisissä MRSA-

tartunnoissa, oireettomilla kantajilla sekä MRSA- ja MSSA-bakteremioissa. Myös spa-tyypin yhteyttä *S. aureus* bakteremian taudinkuvaan analysoitiin.

Tutkimuksen aineistona käytettiin infektioiden seurantatyön tuottamaa tietoa erilaisista rekistereistä. Aineistona käytettiin Tampereen yliopistollisen sairaalan infektioyksikön MRSA-rekisteriä, Pirkanmaan sairaanhoitopiirin käyttämää kaupallista (Neotide) Sairaalan Antibiootti- ja Infektioseurantajärjestelmää (SAI), Pirkanmaan sairaanhoitopiirin alueen potilastietojärjestelmiä, Terveyden ja hyvinvoinnin laitoksen (THL) tartuntatautirekisteriä, hoitoilmoitusrekisteriä (HILMO) sekä sairaalainfektioiden rekisteriohjelmaa (SIRO).

Tutkimuksessa havaittiin MRSA-tartuntoja vuosina 2001-2014 yhteensä 4118 ja näistä 3527 (85.6 %) oli hoitoon liittyviä tartuntoja. Erityisesti pitkäaikaishoitolaitoksissa todettiin runsaasti tartuntoja. Tartunnan alkuperä määritettiin algoritmin avulla, joka huomioi potilaan aikaisemmat hoitojaksot. Epidemian kahtena ensimmäisenä vuotena 71.2 % MRSA-löydöksistä todettiin infektionäytteistä, kun taas tutkimuksen viimeisenä vuonna 2014 vain 9.4 % löytyi infektionäytteistä. Koko tutkimusjakson aikana 78.7 % hoitoon liittyvistä tartunnoista oli saman kannan, t067, aiheuttamia. Epideemisen kannan osuus väheni tutkimusjakson aikana 98.0 %:sta 45.6 %:iin. Uusien MRSA-tartuntojen määrä sairaaloissa kasvoi vuosittain vuoteen 2011 asti, mutta puolittui sen jälkeen. Muiden MRSA-torjuntatoimien ohessa on vuodesta 2011 alkaen seulottu kaikki Pirkanmaan sairaaloihin tulevat potilaat. Seulonnan myötä paljastui lisääntyvästi myös epidemiaan kuulumattomia tartuntoja. Käsihygienian parantumisen merkkinä käsidesinfektionhuuhteen kulutus yli kaksinkertaistui seuranta-aikana.

Vuosina 2005-2015 sekä hoitoon liittyvien että avohoitoperäisten *S. aureus* -bakteremioiden insidenssi kasvoi (21.6-35.8/100 000). Bakteremioista 54.7 % oli hoitoon liittyviä. MRSA-bakteremioiden määrä kasvoi vuoteen 2011 asti ja kääntyi sitten selkeään laskuun. Korkeimmillaan MRSA-bakteremian esiintyvyys Pirkanmaan sairaanhoitopiirissä oli 10-kertainen (4.5/100 000) verrattuna muuhun Suomeen, mutta asettui sittemmin samalle tasolle kuin Suomen muissa sairaanhoitopiireissä (0.6/100 000). MRSA-bakteremioiden väheneminen tapahtui samanaikaisesti MRSA-tartuntojen vähenemisen kanssa. MRSA-bakteremioista 82.6 % oli epideemisen t067 kannan aiheuttamia. MSSA-bakteremoissa spa-tyyppien jakauma oli vaihtelevampaa, eikä yksittäistä vallitsevaa kantaa voitu todeta. Suurin osa MSSA-bakteremioista oli PRSA kantojen aiheuttamia, mutta PSSA:n osuus kasvoi selvästi, 23.9 prosentista 43.1 prosenttiin.

Tutkimuksessa todettiin, että MRSA-bakteremian riskitekijöitä MSSA-bakteremiaan verrattuna ovat edeltävä sairaalahoito, leikkaushoito, tupakointi ja

glukokortikoidihoito. MRSA-bakteremiaan liittyi suurempi kuolleisuus kuin MSSA-bakteremiaan (OR 2.0, 95 % CI 1.20-3.34) ja ero säilyi, vaikka huomioitiin potilaiden ikä, taustasairaudet ja infektiofokus. Infektiofokuksista keuhkokuume lisäsi kuoleman riskiä (OR 2.51, 95 % CI 1.42-4.44) *S. aureus* bakteremiassa, mutta ihoinfektio (OR 0.42, 95 % CI 0.24-0.74) tai luuinfektio (0.32, 95 % CI 0.11-0.90) liittyivät pienempään kuoleman riskiin. Myös edeltävä glukokortikoidihoito, maksakirroosi, korkea ikä, sydänsairaus tai infektiofokuksen jääminen epäselväksi suurensivat kuolemanriskiä. Bakteerikantaan tehoavan empiirisen antibiootin ei voitu tässä tutkimuksessa osoittaa vaikuttavan kuolleisuuteen.

Tapauskuolevuus laski vuodesta 2005 (21.8%) vuoteen 2015 (17.1 %). Korkein kuolleisuus todettiin spa tyyppien t002 ja t067 aiheuttamissa bakteremioissa (27.6 % ja 26.9%). Klonaalisista komplekseista suurin kuolleisuus liitty spaCC 067-ryhmään (25.6%). Tutkimuksessa saatiin myös viitettä siitä, että spaCC 008-ryhmä liittyisi muita ryhmiä useammin endokardiittiin, spaCC 012 vierasesineinfektioihin ja spaCC 2133 ihoinfektioihin.

Yhteenvetona todetaan, että sekä uudet hoitoon liittyvät MRSA-tartunnat että MRSA-bakteremiat vähentyivät Pirkanmaan sairaanhoitopiirissä vuosina 2001-2014. Muutos selittyy parhaiten tehostuneella hoitoon liittyvien infektioiden torjunnalla. Koska epidemian synty ja laantuminen liittyvät t067-kannan muutoksiin, on mahdollista, että myös bakteerikantojen luonnollisella vaihtelulla on osuutta. Tehostuneesta hoitoon liittyvien infektioiden torjunnasta huolimatta antibiooteille herkkien *S. aureus* -kantojen aiheuttamat bakteremiat lisääntyivät. Tämä johtunee ennen kaikkea tehostuneesta diagnostiikasta, mutta myös väestön ikääntymisestä ja lisääntyneistä lääketieteellisistä toimenpiteistä. PSSA:n suhteelliseen lisääntymiseen syy on epäselvä. Mitään yksittäistä epideemistä kantaa lisääntyneiden PSSA bakteremioiden taustalla ei todettu. Uutena löydöksenä todettiin tupakoinnin ja glukokortikoidihoidon altistavan MRSA bakteremialle.

Tapauskuolevuuden lasku viittaa parantuneeseen hoidon tasoon. Myös tehostunut diagnostiikka saattoi tuoda esiin lievempiä tautitapauksia, joiden ennuste on parempi. Kuten aikaisemminkin on havaittu, MRSA-bakteremia on tappavampi tauti kuin MSSA-bakteremia. PRSA ja PSSA bakteremioiden välillä ei todettu tilastollisesti merkitsevä eroa taudinkuvassa tai kuolleisuudessa, joskin trendi viittaa siihen, että PRSA bakteremioissa kuolleisuus saattaa olla hieman suurempi. Epäselväksi jäänyt infektiofokus lisäsi kuoleman riskiä, mikä korostaa potilaan perusteellisen tutkimisen tärkeyttä. Suurin kuolleisuus liittyi spa tyyppeihin t002 ja t067 sekä kompleksiin spaCC 067. Spa t067 liittyy lähes aina MRSA-ominaisuuteen, mikä osaltaan selittää tähän kantatyyppiin liittyvän korkean kuolleisuuden. Lisäksi

t067 oli erityisen tavallinen löydös pitkäaikaishoitolaitoksissa, joissa potilaiden ennustetta bakteremian yhteydessä heikentää lukuisat taustasairaudet ja korkea ikä. Sekä t002 että t067 kuuluvat kuitenkin samaan klonaaliseen kompleksiin mikä saattaa viitata siihen, että näillä sukulaiskannoilla on jokin yhteinen virulenssiominaisuus, joka liittyy vaikeampaan taudinkuvaan.

MSSA-bakteremioissa spa-tyyppijakauma oli hyvin heterogeeninen. MRSA-bakteremioissa puolestaan t067 oli yleisempi kuin seulontanäytteissä viitaten bakteerin kykyyn aiheuttaa invasiivisia infektioita. Tämän kannan laaja levinneisyys Pirkanmaalla sekä saman kannan laaja esiintyminen Espanjassa viittaavat siihen, että kannalla on myös tehokasta leviämistä edistäviä tekijöitä. Spa tyyppi t1012 oli hyvin yleinen seulontanäytteissä, mutta ei aiheuttanut MRSA-bakteremioita Pirkanmaalla. Tämäkin tukee sitä näkemystä, että *S. aureus* kannat eroavat leviämis- ja taudinaiheuttamiskyvyltään. Tutkimuksessa todettiin spaCC 067, 008 ja 012 ryhmien liittyvän tiettyihin bakteremian fokuksiin ja vaikeaan taudinkuvaan.

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals. Also some additional data has been included.

I. Jokinen E, Laine J, Huttunen R, Arvola P, Vuopio J, Lindholm L, Vuento R, Syrjänen J: Combined interventions are effective in MRSA control. Infectious Diseases Vol 47:11 pp. 801-807 (2015).

II. Jokinen E, Laine J, Huttunen R, Rahikka P, Huhtala H, Vuento R, Vuopio J, Syrjänen J: Comparison of outcome and clinical characteristics of bacteremia caused by methicillin-resistant, penicillin-resistant and penicillin-susceptible *Staphylococcus aureus* strains. Infectious Diseases Vol 49:7 pp. 493-500 (2017)

III. Jokinen E, Lindholm L, Huttunen R, Vuento R, Vuopio J, Syrjänen J: Spa type distribution in MRSA and MSSA bacteremias and association of spa clonal complexes with the clinical characteristics of bacteremia. Eur J Clin Microbiol Infect Dis. 2018 Feb 10. doi: 10.1007/s10096-018-3210-1. [Epub ahead of print].

IV. Jokinen E, Laine J, Huttunen R, Lyytikäinen O, Vuento R, Vuopio J, Syrjänen J: Trends in incidence and resistance patterns of *Staphylococcus aureus* bacteremia. Infectious Diseases Vol 50:1 pp. 52-58 (2018)

ABBREVIATIONS

BURP	based upon repeat pattern
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ECDC	European Centre of Disease Prevention and Control
CA	community-acquired
CC	clonal complex
CDC	Centers for Disease Control and Prevention (USA)
CI	confidence interval
CLSI	Clinical and Laboratory Standards Institute
CO	community onset
CVC	central venous catheter
D28	day 28
D90	day 90
HA	health care associated
HILMO	National Hospital Discharge Database
HD	hospital district
HO	hospital onset
ICU	Intensive Care Unit
IC-nurse	infection control nurse
ID-specialist	infectious diseases specialist
IU	Infectious Disease Unit
LTF	long-term facilities
MIC	minimum inhibitory concentration
MRSA	methicillin-resistant *Staphylococcus aureus*
MSSA	methicillin-susceptible *Staphylococcus aureus*
MLST	multilocus sequence typing
NIDR	National Infectious Diseases Register
OR	odds ratio
PCR	polymerase chain reaction
PE-MRSA	Pirkanmaa epidemic MRSA strain

PRSA	penicillin-resistant *Staphylococcus aureus*
PSSA	penicillin-susceptible *Staphylococcus aureus*
PFGE	pulse-field gel electrophoresis
PVL	Panton-Valentine leukocidin
SIRO	Finnish National Surveillance Program of Blood Stream Infections
SAI	Commercial Surveillance System for Antibiotics and Infections
S. aureus	*Staphylococcus aureus*
SAB	*S. aureus* bacteremia
SCCmec	staphylococcal cassette chromosome mec
SH	secondary care hospital
Spa	Staphylococcal protein A
SSTI	skin and soft tissue infections
ST	sequence type
TAUH	Tampere University Hospital
THL	National Institute for Health and Welfare

1 INTRODUCTION

Staphylococcus aureus (S. aureus) is a pathogen that poses a remarkable burden to the population. The variety of diseases caused by *S. aureus* range from mild local skin infections to fatal sepsis. Serious forms of *S. aureus* disease are associated with significant morbidity and mortality. The mortality rate in *S. aureus* bacteremia (SAB) in the pre-antibiotic era was 80% [1]. The introduction of antibiotics in the 1940s and 1950s resulted in better outcomes. Thereafter, a greater understanding of SAB management has improved outcomes even further [2]. Still the mortality rates per population are comparable to those associated with breast cancer and prostate cancer. In the USA it has been estimated, that SAB associated mortality is higher than mortality associated with AIDS, tuberculosis and viral hepatitis combined [3]. Despite high mortality rates, the impact of SAB in the community remains underestimated.

Emerge of methicillin-resistant *S. aureus* (MRSA) strains have made the management of *S. aureus* diseases even more challenging. The european Centre of Disease Prevention and Control (ECDC) estimated in 2008 that annually the total number of health care associated MRSA (HA-MRSA) infections in the member states of European Union, Iceland and Norway was 171 000 resulting in 5400 excess deaths [4].

Until recent decades, MRSA was rare in Finland. A large MRSA epidemic broke out in Pirkanmaa county in 2001 and rapidly spread to several health care institutions [5]. To control the epidemic, several infection control improvements were made during the recent years. This exceptional epidemic provided an opportunity to observe the influence of hospital hygiene interventions on the incidence of MRSA transmission and infections. Surveillance of the efficacy of control efforts is essential to guide their use and investments on them [6]. As the majority of the *S. aureus* disease burden comes from methicillin-susceptible strains (MSSA) it is relevant to also assess the impact of improved hospital hygiene on MSSA infection prevention.

Relatively high occurrence of MRSA bacteremias in the county made it possible to study differences in risk factors and clinical course of MRSA and MSSA bacteremias. Although several other laboratories have discontinued the testing of

penicillin susceptibility, the central laboratory of Pirkanmaa, Fimlab, has continued to do so. This provided an opportunity to explore the clinical course and trends in the incidence of bacteremias caused by the subgroubs of MSSA, namely penicillin-susceptible (PSSA) and -resistant (PRSA) *S. aureus.*

The purpose of MRSA strain typing is to yield data for epidemiological surveillance and infection control. New MRSA findings and invasive strains are routinely typed as a part of continuous surveillance, but the data on MSSA strain distribution is scarce. Understanding the epidemiology of MSSA is important because MSSA strains are the reservoir where MRSA emerge [7,8]. Staphylococcal protein A (spa) that is used for strain typing in Finland also has virulence properties and may impact the disease course. There is only very little data on its association to the clinical charactersitis of SAB [9].

The objectives of this study were to study MRSA transmissions and SAB incidence in Pirkanmaa county and the potential impact of improved hospital hygiene on these. We also observed the trends in antibiotic resistance and factors associated with clinical characteristics and mortality in SAB. Spa type distribution in MRSA transmission, MRSA bacteremias and MSSA bacteremias was studied and its association to clinical course of SAB was analysed.

2 REVIEW OF THE LITERATURE

2.1 Health-care associated infections

The distinction between community-acquired (CA) and health care associated (HA) infections is important for the guidance of infection control strategies as their reduction requires different strategies. Interventions in health care are mostly addressed to reduce HA infections. The burden of HA infections on healthcare facilities also needs to be assessed. HA infections lead to increased mortality, morbidity, prolonged hospital stays and increased costs. It has been estimated that the risk ratio for death attributable to HA infections in hospitalized patients is 2.6-3.52, the extension of length of hospital stay is 13.0-19.9 days per patient and costs are 15 400 euros per patient [10]. Harbarth et al have estimated in a systematic review, that at least 20%, but in some setting even up to 70%, of health-care associated infections are preventable [11].

2.1.1 Definition

The widely accepted Centers for Disease Control and Prevention (CDC) guidelines define HA infections as localized or systemic condition resulting from an adverse reaction to the presence of an infectious agent(s) or its toxin(s) which occurs on or after the 3rd calendar day of admission to an inpatient location where day of admission is calendar day 1. There should be no evidence that the infection was present or incubating at the time of admission to the hospital. The infection may arise from either endogenous or exogenous source [12].

Erb et al studied the transmission chains of patients who met CDC criteria for HA using molecular strain typing methods [13]. They criticized that the CDC definition overestimates the proportion of true HAs. However, CDC guidelines are a pragmatic tool for surveillance of large quantities of patients.

Finnish national surveillance program of blood stream infections (SIRO) gives precise guidelines to judge the setting of a bacteremia: Bacteremia is defined as at least one positive blood screen associated with signs and symptoms of an infection.

It is defined HA if the symptoms started more than 48 h after hospital admission or the bacteremia was related to surgical operation within the past 30 days or other invasive procedure within the last 10 days. Patients on chronic renal replacement therapy or neutropenic due to treatment of malignancy are classified as having a HA bacteremia [14].

Diverse terminology has been used in literature to describe potential association of infections and health care settings. Nosocomial infection has previously been used instead or as a synonym of HA infections in CDC guidelines. Also the term hospital-acquired infection has been used widely and as a synonym of HA, but literally taken it excludes infections acquired in ambulatory health care. Some studies use terms hospital-onset (HO) or community-onset (CO) bacteremia. HO-bacteremia is defined as an infection with positive blood culture obtained 48 hours post admission or within two days of discharge [15]. Infection may also be classified as health care associated with community onset distincting this subgroup from absolute community associated infections with no previous health-care contact.

2.1.2 Significance of *Staphylococcus aureus* in health care associated infections

S. aureus is among the most common causes of HA infections worldwide and accounts for approximately 13-26% of all HA bacteremias in Finland [16-19]. Hospitals offer favourable environment for *S. aureus* to spread and cause diseases. Symptomless *S. aureus* colonization readily progresses to infection when skin or mucous barrier is compromised for example due to trauma, surgical procedure or vein puncture [20]. Patient to patient transmissions also occur, usually mediated by staffs' contaminated hands or shared facilities such as toilets and bathrooms. Especially patients with chronic skin ulcers can shed large quantities of bacteria to their environments. In a study examining the survival of bacteria on various synthetic materials used in hospitals, *S. aureus* was able to survive for weeks [21]. Infectious doses of MRSA have also been reported to be relatively small compared to other common pathogens, approximately four colony forming units [22].

2.2 *Staphylococcus aureus*

2.2.1 Characteristics of *Staphylococcus aureus*

S. aureus, a member of a *micrococcae* family, is a Gram-positive coccus-shaped bacterium. It is coagulase- and catalase-positive facultative aerobic and can be identified from its typical β-hemolytic, yellow pigmented and large colonies on blood agar. However, all *S. aureus* colonies do not have typical yellow appearance and small colony variants, characterized by small colony size and slow growth, may lead to misidentification of *S. aureus* [23]. Coagulase activity and latex agglutination test can be used to distinguish *S. aureus* from coagulase negative staphylococcae also commonly found in clinical samples [24]. As conventional identification methods rely on lengthy cultures, mass spectrometry is nowadays used for the identification of *S. aureus* [25].

S. aureus is a human commensal but also an important pathogen causing wide range of infections from mild local skin infections to life threatening invasive infections. The spectrum of staphylococcal diseases includes skin and soft tissue infections, bone, joint and respiratory tract infections and infections related to foreign bodies, such as prosthetic valves and joints and pace makers. It is also a common cause of venous catheter associated infections. Abscess formation is typical for *S. aureus* infections. It also causes toxin-mediated diseases, such as food poisoning, necrotizing pneumonia and toxic shock syndrome. The most serious form of *S. aureus* disease is septic shock and endocarditis [20].

2.2.2 Antibiotic resistance of *Staphylococcus aureus*

S. aureus is naturally susceptible to several antibiotics but has the ability to acquire resistance by horizontal transfer of resistance genes from outside sources [26]. Acquisition of new genetic material by antimicrobial-susceptible bacteria from resistant strains is aided by gene transfer systems, such as plasmids and transposons [27]. Also, chromosomal mutation and antibiotic selection pressure play a role in the development of antibiotic resistance. However, the evolutionary processes that lead to the development of resistant strains proceed slowly. Individuals´ carriage of a resistant bacterium usually results from transmission of a clonally spreading resistant strain.

First reports of penicillin resistance came out a few years after the introduction of penicillin [28,29]. These PRSA strains produce a penicillinase-enzyme that hydrolyses the beta-lactam ring of penicillin essential for its antimicrobial activity. By 1950s PRSA strains had became pandemic. Epidemiological data from the 1990s reported that PSSA strains were very rare (<5%) and replaced by PRSA strains [20]. Consequently, many laboratories discontinued routine testing of penicillin susceptibility [30]. However, based on more recent data from Europe, 14-35 % of MSSA isolates from nasal swabs [31] and 31% of invasive strains were susceptible to penicillin [30]. Also, Chrane et al have reported rising PSSA rates in New York up to 13-15% [32]. Distinction between PSSA and PRSA strains is reasonable bearing in mind the advantages of penicillin treatment. Penicillin treatment in PSSA bacteremia is shown to be more effective than cefuroxime [33] and is better tolerated than cloxacillin [30].

Methicillin, a penicillin derivative that was resistant to beta-lactamase hydrolysis was invented in 1959. The first *S. aureus* strains resistant to methicillin were found in 1961 in the United Kingdom [34]. This was followed by several hospital outbreaks of MRSA in 1970s leading eventually to the global pandemic that continues to the present times [26].

Methicillin resistance differs in mechanism from penicillin resistance. Resistance determinant, *mec* gene, is located in a mobile genetic element, staphylococcal cassette chromosome mec (SCCmec) [35]. The emergence of methicillin-resistant staphylococcal lineages is due to the acquisition and insertion of the SCCmec element into the chromosome of susceptible strains. Thirteen SCCmec types have been identified to date [36]. *Mec* gene encodes penicillin binding protein (PBP2a) that has a low affinity to beta-lactam antibiotics [37]. This leads to broad resistance to beta-lactams: penicillin, cephalosporins and carbabenems, making this mechanism clinically relevant. Two types of *mec* genes have been identified so far, *mecA* and *mecC,* of which *mecA* is more widely distributed. Carriage of *mec* gene can be detected using polymerase chain reaction (PCR) [38].

S. aureus strains that are resistant to methicillin are usually treated with vancomycin. Vancomycin resistance has been rare so far [39]. It is thought to arise from acquisition of resistance determination gene from enterococci. The mechanism underneath development of vancomysin-intermediate strains is different and associated with changes in the bacterial cell wall synthesis and metabolic pathways. These changes increase the minimum inhibitory concentration (MIC) of the bacterium and affect the attainable antimicrobial plasma

concentration necessary to reach bacteriocidal effect. Increased MIC-value is associated with poorer treatment outcome [40].

2.2.3 *Staphylococcus aureus* carriage

S. aureus is a human commensal that can be cultured from multiple sites of the skin and mucosal surfaces of carriers. Nostrils are thought to the primary reservoir [41]. Molecular determinants of carriage are not fully understood but mucin appears to be the most important host surface that is colonized [20]. The process involves interactions between staphylococcal protein and mucin carbohydrates and bacterial adhesins. Host´s HLA genotype and previous viral infections may also play a role in it [41].

Three patterns of *S. aureus* carriage have been distinguished. It has been estimated that 20% of healthy individuals are persistent *S. aureus* carriers, 60% are intermitted carriers and 20% are non-carriers. Individuals´ carrier pattern may change over the years [41].

2.2.4 Risk of *Staphylococcus aureus* carriage

Kluytmans et al reported that chronic renal replacement therapy, insulin-dependent diabetes mellitus, intravenous drug use and HIV-infection were risk factors for *S. aureus* carriage [41]. Another study reported that *S. aureus* nasal carriage is more common in males and inversely correlated with age. In that study, exposure to health care environments was not a risk factor for *S. aureus* carriage but it was a risk factor for MRSA nasal carriage [17]. Contact sports increase the risk of both *S. aureus* and MRSA carriage [42]. In children, having a family member working in a hospital increases the risk of nasal MRSA colonisation [43]. Both occupational exposure to livestock or living on a livestock farm are known risk factors MRSA carriage [44]. One study reported that younger age and antibiotic exposure may increase the risk of nasal carriage of resistant *S. aureus* [45]. Having a MRSA colonized family member is also a risk for MRSA colonization [46,47]. Other reported risk factors for MRSA carriage are older age, pressure ulcers and prolonged stay at an intensive care unit (ICU), intravascular devices, mechanical ventilation, multiple antibiotic therapy and tracheostomy [48].

In conclusion, *S. aureus* carriage is more common in young and in male but is also associated with diabetes, dialysis, intravenous drug use and HIV. MRSA

carriers are usually exposed to health care environments or livestock either directly or via their close contacts. Both young and old people are at higher risk of MRSA colonisation than middle aged.

2.2.5 Prevalence of *Staphylococcus aureus* carrige

In a population-based study performed in Germany, the prevalence of *S. aureus* nasal carriage was 27.2% (30.0% in males and 24.3% in females) and the prevalence of MRSA nasal carriage was 0.34% [17]. In a cross-sectional cohort study, the overall prevalence of *S. aureus* carriage was reported to be 21.6% in Europe [49]. Nine countries were included in the study and carriage ranged from 12.1% in Hungary to 29% in Sweden. Overall prevalence of MRSA carriage was low, 0.3% and ranged from 0,0% in Sweden to 0.4% in Belgium, Croatia, France, and the UK. In children in the USA, increasing *S. aureus* and MRSA nasal carriage prevalence up to 36.4% and 9.2%, respectively, was reported [43]. In a meta-analysis by Salgado et al, the prevalence of *S. aureus* colonization ranged from 22.8% to 36.2% and MRSA colonisation from 0.2 to 7.4% in community dwelling people [46].

In Finland, the prevalence of *S. aureus* carriage has been studied in nursing homes by taking screening swabs from non-infected residents. Forty-four per cent of residents were *S. aureus* carriers and 0.9% MRSA carriers [50].

National Institute for Health and Welfare (THL) collects notifications of new MRSA findings in Finland. Each subject is notified only once at the time of the initial finding, regardless of later findings. A positive MRSA sample may be derived either from a screening sample or from clinical sample. The incidence of MRSA findings has increased significantly over two decades. In 1996, the incidence was 2.25 per 100 000 population and in 2016 it was 31.11 per 100 000 population [51]. The observed incidence obviously depends on screening activity.

In conclusion, point prevalence studies have shown that about one third of population are at least transient *S. aureus* carriers but the prevalence of MRSA carriage is low in Europe. MRSA notifications have increased significally in Finland during the last 20 years.

2.2.6 Duration of *Staphylococcus aureus* carriage

MRSA carriage can subside either spontaneously or due to eradication therapy [52]. In a Swedish retrospective study, 535 MRSA colonized patients, both HA and CA, were followed for four years [53]. The median duration of carriage after detection of MRSA carrier status was 179 days, but 43% cleared the MRSA colonization in less than 2 months. Having a household member with MRSA colonization, young age (0-17 years), spa-type t002 and colonization in two or more locations, were associated with a longer colonization phase. Antibiotic treatment for clinical infection and decolonization therapy was significantly associated with a shorter carriage time [53]. In a study by Marshall et al, 58.6 % of MRSA carriers cleared colonization within a medium time of 7,4 months [54]. Factors associated with prolonged carriage were use of antibiotics, indwelling devices, presence of a skin lesion, immunosuppressive therapy and hemodialysis. Chronic skin lesions have also been associated with persistent MRSA carriage [55]. In a study by Cluzed et al, the median duration of MRSA colonization after CA SSTI caused by MRSA was 21 days, but a significant proportion of patients (19.8%) remained colonized at the end of the 6 months follow up. Treatment with clindamycin was associated with shorter colonization and increased age was associated with longer duration of colonization [56] .

In conclusion, in the majority of patiens, MRSA colonization may resolve in six months, but longer carriage is not uncommon. Subjects with chronic illnesses, skin lesions and colonized household member are at the greatest risk of acquiring long-term MRSA colonization.

2.2.7 *Staphylococcus aureus* carriage and risk of infection

Carriage of *S. aureus* has been identified as a risk factor for the development of HA infections in both surgical and non-surgical settings [48,57,58]. The combined data from four studies in the 1990s gives a relative risk of 7.1 (95% confidence intrerval (CI) 4.6 to 11.0) for surgical infection in *S. aureus* carriers versus non-carriers [41]. In major heart surgery, *S. aureus* nasal carriers had a significantly higher risk of surgical site infection than non-carriers (12.5% and 5.0%, respectively) [59]. A meta-analysis showed an increased risk of surgical site infection in orthopedic surgery in patients with *S. aureus* nasal carriers (odds ratio (OR) 5.92, 95% CI 1.15-30.39) [60]. Relative risk of *S. aureus* infection between carriers and non-carriers in

haemodialysis ranges from 1.8 to 4.7 [41]. In a study by Pujol et al, 84% of patients with nosocomial SAB in ICU had prior nasal *S. aureus* carriage [48].

Risk of MRSA infections following MRSA carriage is thought to be even greater than the risk of MSSA infection following MSSA carriage. Pujol et al reported in a prospective study that the relative risk for SAB was 3.9 (95% CI 1.6-9.8) for MRSA carriers compared with MSSA carriers in ICU patients. The analysis was adjusted with the presence of intravascular catheters and Simplified Acute Physiologic Score, which reflects the patients´ clinical condition [48]. Risk of surgical site infection after heart surgery in patients with MRSA carriage was higher than in patients with *S. aureus* carriage (33.0% versus 12.5%, respectively) [59]. In long-term facilities (LTF), the relative risk of developing a staphylococcal infection due to MRSA vs MSSA was 3.8 (95% CI, 2.0 to 6.4) [61]. MRSA carriage is also associated to with an increased risk of hospital readmissions [62]

Strains colonizing and causing invasive infections are often identical [41,57,61] . This, in addition to the association of *S. aureus* carriage and infection risk, shows that *S. aureus* infections have in most cases their origin in the patients' endogenous flora.

2.2.8 *Staphylococcus aureus* strain identification

The purpose of strain identification is to yield data for epidemiological surveillance and infection control. Laboratory-based surveillance monitors the baseline occurrence of different strain types and transmission chains responsible for outbreaks. In the presence of increased infection rates strain typing can reveal clonal relatedness among the strains, which may suggest a common source. If it appears that several unrelated strains are found behind increased infection rates, it might be reasonable to focus on improving general infection control strategies [41].

Pulsed-field gel electrophoresis (PFGE) has been a gold standard for strain typing of MRSA, although it is time-consuming and the inter-laboratory comparability of results is challenging. Therefore, DNA sequence-based methods are now more frequently used. In Finland, spa typing replaced PFGE as the primary method for MRSA strain typing in 2009 [63]. Good concordance between these methods has been shown especially regarding the most common strain types found in Finland [5,63]. Spa typing is based on single-locus DNA sequencing of the polymorphic region of the *S. aureus* protein A (*spa*) gene [64]. The method is reproducible, has high discrimination power and is faster than PFGE and provides unambiguous data for international comparison [63]. Mutations in the *spa* gene may

also occur during outbreaks and falsely indicate that the outbreak has several sources instead of a single source. Related *spa* types can be grouped to spa clonal complexes (spaCC) according to a repeat structure using an algorithm [65]. At present, the most accurate method for strain distinction is whole-genome sequencing [66].

In many studies reporting genetic distribution of MRSA lineages multi-locus sequence typing method (MLST) is used. MLST method is based on the systematic sequencing of seven well-conserved house-keeping genes within the bacterial genome and allows classification of *S. aureus* strains into sequence types (ST) [67]. Closely related STs can be further grouped into clonal complexes (CC) by a BURST algorithm [68]. MLST is more expensive and has lower discriminatory power compared to PFGE and is not thus suitable for routine surveillance of *S. aureus* epidemiology [64]. However, is seems that *spa* typing produces results that are comparable with those produced by MLST [69]. The Ridom spa server can be used to map specific spa types to known MLST designations [70].

In Finland, MRSA strain from each newly recognized MRSA carrier is spa typed and saved to MRSA database by THL. Spa typing is also done each time when MRSA is recovered from blood cultures. MSSA strains are not routinely typed.

2.3 *Staphylococcus aureus* bacteremia

2.3.1 From carriage to infection

The chain of events during the progression from *S. aureus* carriage to disease is not fully established. An infection may follow the invasion of colonizing bacteria into the bloodstream through damaged epithelia, but also changes in the host–pathogen interaction may contribute to the process. The interplay between *S. aureus* virulence properties and host defence mechanism determines whether an infection is restricted or progresses [20]. Risk of infection is greater in the presence of foreign material, which is associated with impaired local immunodefence [71]. Also, the immunocompromized state resulting from treatment of autoimmune diseases may increase the risk [72,73]. The endothelial cell is thought to have a central role in the pathogenic processes because staphylococci are phagocytized by endothelial cells after adherence. The intracellular environment protects staphylococci from host defence mechanisms and antibiotics [20].

Genetic factors of the microbe are also important in the progression from carriage to infection. Young et al have demonstrated in a single patient that only eight mutations in the genome of *S. aureus* accompanied the transition from carriage to invasive infection [66]. The mutations caused changes in proteins that regulate virulence determinants of the bacteria.

2.3.2 Incidence of *Staphylococcus aureus* bacteremia

S. aureus is among the most common causes of bacteremia and it is more common in males and in aged population [74-76]. Studies from the Nordic countires have reported increase in the incidence of SAB. In the years 1981-2000 an increasing incidence from 18.2 to 30.5 cases/100 000 population was observed in Denmark [77]. Annual increase in incidence rates was more pronounced in CA bacteremias compared to HA bacteremias (6.4% vs 2.2%). Asgeirsson et al reported increasing incidence between 1995-2008 in Iceland from 22.7 to 28.9 per 100 000. The increase constituted of CA bacteremias since the incidence of HA cases declined [78]. Norway reported increase in SAB incidence from 24/100 000 to 27/100 000 between years 2002 and 2013 [79].

A large, multinational stydy by Laupland et al included 18 430 SAB episodes in Europe [75]. The overall annual incidence rate for SAB was 26.1, for MSSA 24.2 and for MRSA 1.9 per 100 000 population. The incidence rates of hospital-onset MSSA (9.2 per 100 000), community-onset MRSA (1.0 per 100 000) and hospital-onset MRSA (0.8 per 100 000) bacteremia varied substantially across regions while the incidence rate of community onset MSSA bacteremia was similar across regions (15/100 000). The overall incidence of SAB did not increase within the study period (years 2000-2008), but the proportion of MRSA bacteremias increased. This contradicts findings by Mostofsky et al who have earlier concluded in their review that the burden of MRSA bacteremias does not replace MSSA but adds to it [80]. However, in Laupland´s study there was significant variation in incidence of MRSA within regions and MRSA appears to cause replacement in some regions and additive effects in others [75]. The proportion of CA bacteremias increased according to this study, which is in line with studies from Denmark and Iceland. A population-based stydy from New Zealand reported a stable incidence of SAB [76].

Incidence of SAB in Finland was studied in 1995-2001 [81]. An increasing incidence from 11/100 000 to 17/100 000 was observed. Proportion of HA infections remained stable, 51%. Finland was also included in the multinational study by Laupland et al [75]. When gender and age adjusted incidence in 2000-2004

was compared to the incidence in 2005-2008, an increase from 19.0 to 21.8/100000 was observed (incidence rate ratio 1.15, 95% CI 1.10-1.19) A slight increase was seen in all other subgroups except in CA-MSSA bacteremias which remained stable (11.9/100 000 CA-MSSA, 6.5-9.0/100 000 for HA-MSSA, 18.9-21.1/100 000 for MSSA, 0.09-0.2/100 000 for CA-MRSA, 0.1-0.3/100 000 for HA-MRSA and 0.2-0.6/100 000 for MRSA). The number of MSSA bacteremia cases reported to National Infectious diseases Register (NIDR) at the THL increased from 992 in 2005 to 2013 in year 2015. The number of reported MRSA bacteremia cases was 27 in year 2005 and 41 in year 2015 [51].

Proportion of penicillin susceptible strains in SAB has been studied less extensively. Cheng et al have reported 28% proportion of PSSA in MSSA bacteremias in Canada [82] and Resman et al have reported 31% of PSSA in MSSA bacteremias proportion in Sweden [30].

In conclusion, incidence of SAB is either stable or increasing and the potential increase constitutes of the emergence of MRSA and increase of CA bacteremias in most ares. However, there is significant regional variation in the incidences. A significant part of MSSA bacteremia isolates appears to be susceptible to penicillin.

2.3.3 Outcome of *Staphylococcus aureus* bacteremia and effect of antibiotic resistance

SAB is the most severe form of *S. aureus* infection and it is associated with significant mortality. Population-related mortality in SAB is higher than in bacteremia caused by other pathogens [79,83].

A recent population-based study from Denmark reported that 30-day mortality was 24.7% in community-acquired SAB [84]. The proportion of MRSA bacteremias was 0.5% in the study. Day 28 mortality in Finland was reported by Lyytikäinen et al in 1995-2001 and it was 17% [81]. At that time, no MRSA bacteremias had been diagnosed in Finland.

Yahav et al compared time trends in 30-day mortality of SAB in tertiary centre in years 1988-2010. They found increasing mortality rate up to 42.9% over time. Also, increasing age and co-morbidities of SAB patients was noted, which probably explains the increased mortality [85]. Proportion of MRSA was high (approximately 30%) and did not vary significantly within the study period.

On the contrary, several studies have reported decreasing mortality trends [77,78,86]. Reports of decreasing mortality rates are mainly from Scandinavia where MRSA rates are low. Lowest 30-day mortality was reported in Iceland, 8.1% [78].

Several studies have analysed the differences in mortality between MSSA and MRSA bacteremia. Meta-analysis including 3963 bacteremia patients concluded, that MRSA bacteremia was associated with higher in-hospital mortality (OR, 1.93; 95% CI, 1.54-2.42) [87]. This association persisted even when adjusted for comorbidities or severity of illness. However, the majority of studies referred to in the meta-analysis did not find difference in MSSA and MRSA bacteremia mortality. The authors stated that these studies probably lacked power due to too small sample size. A more recent study by Kang et al confirmed the previous findings that MRSA bacteremia was significantly associated with mortality in multivariate analysis (Adjusted OR = 1.69, 95% CI 1.15-2.49) [88].

The effect of methicillin-resistance on the long-term outcome of SAB patients has also been studied [89]. The crude survival time of patients with MRSA bacteremia was shorter than it was for patients with MSSA bacteremia (14 months (IQR 1-86) vs 54 months (IQR 3-105); hazard ratio 1·46, 95% CI 1·18-1·79; p=0·01). However, in multivariate analysis with confounding factors the strength of the association between MRSA bacteremia and survival diminished and authors concluded, that MRSA patients´ other poor prognostic factors may completely explain the shorter survival.

Studies comparing differences in clinical course of PRSA and PSSA are scarce probably due to the underestimated prevalence of penicillin susceptibility. No significant difference in mortality between PSSA and PRSA has been reported [82,90].

In conclusion, SAB is associated with significant mortality. Crude mortality associated with MRSA is significantly higher than in MSSA bacteremias, but this can largely be explained by confounding factors such as underlying conditions.

2.3.4 Focus of the infection in *Staphylococcus aureus* bacteremia and its association with the outcome

Jabobssen et al reported in their prospective, population based study, that the most common focus of infection in invasive *S. aureus* disease (mostly bacteremias) was skin and soft tissue infections (SSTI) (19%) [91]. Other most common foci were arthritis (14%) and line-associated infection (14%). The percentages of patients with endocarditis, osteomyelitis (other than vertebral), vertebral osteomyelitis and respiratory infection were 6%, 8%, 4% and 5%, respectively. In 19% of the patients the focus remained unknown. Arthritis and osteomyelitis (other than

vertebral) were associated with better prognosis and endovascular focus (mainly endocarditis), respiratory infection and unknown focus with a worse outcome.

A pooled analysis of five studies gave consistent results [92]. Intravenous catheters were the most common identified focus (27.7%). Other common foci were SSTI (14.8%), endocarditis (8.3%) and pneumonia (5.2%). Focus remained unknown in 18.9% of the patients. Pneumonia, endocarditis and unknown focus were associated with mortality. Relation of unknown focus was discussed to result from insufficient investigations to identify infective focus and may thus lead to insufficient treatment and source control. However, patient with unidentified focus were also older and some of them died before proper focus investigations had been performed. Line-associated bacteremia may be related to lower mortality rate [93].

Some studies have compared differences in infective focus between MRSA and MSSA bacteremia. MRSA bacteremia patients were shown to have less endocarditis than MSSA patients (11% vs 5%, respectively) [89]. Wang et al studied infectious foci in CA-MRSA and CA-MSSA bacteremias in Taiwan [94]. SSTI and necrotizing pneumonia were more common foci in MRSA bacteremias and endocarditis in MSSA bacteremias. No difference in distribution of other foci was found.

Differences in the occurrence of infective focus between HA and CA have also been reported [95]. Unknown focus and SSTI are common in CA bacteremias while surgical site infections and catheter associated infection are common in HA infections.

In conclusion, SSTI, central line associated, endocarditis, pneumonia and osteomyelitis are common foci of SAB. Endocarditis, pneumonia and unknown focus are associated with worse prognoses.

2.4 Factors associated with the severity of *Staphylococcus aureus* bacteremia

In addition to antimicrobial resistance, many other factors such as host chracteristics, setting of the bacteremia, virulence properties of the bacterium and treatment affect the outcome of SAB.

2.4.1 Host factors

Several host factors are known to associate with the severity of the SAB [3,96-99]. Mortality within 30 days has been shown to be higher in female patients (29% vs. 22%) with an adjusted hazard ratio of 1.30 (95% CI, 1.11-1.53) [84]. Malani et al suggested that for every decade of increase in age, the odds of death within six months of SAB doubles [96]. They also found chronic kidney insufficiency to be associated with increased mortality. A review by van Hal et al concluded that age is an independent risk factor and the risk associated with aging is directly linked to changes within the host as a consequence of the aging process and it is not explained solely by confounding diseases. There is no clear evidence that ethnic background or socioeconomic status would influence the outcome [3]. Obesity is associated with poor prognosis in patients with bacteraemia but its impact on SAB mortality *per se* has not been studied [100].

Outcome of SAB in immunocompromised patients has been studied infrequently probably due to the heterogeneity of these disorders [101]. Forsblom et al found glucocorticoid therapy and immunosuppressive treatment to have negative prognostic impact [98]. Surprisingly, neutropenic patients due to cancer treatment are less likely to die of SAB [99] . It has been discussed that immunosuppression may even be protective in some cases, as dampened host responses may lead to reduced inflammation, manifesting as less severe disease [99,101]. In neutropenic, hospitalised patients also the rapid onset on treatment may improve the oucome [99]. No studies have detected higher mortality among HIV patients [101]. Forsblom et al found age, chronic alcoholism and immunosuppressive treatment to be associated with mortality on day 28 (D28) [97]. Also, liver cirrhosis, congestive cardiac failure, malignancy, hemodialysis and the presence of multiple comorbidities have been reported to increase the risk of death in SAB [101].

In conclusion, female sex, serious underlying diseases and older age are important host dependent predictors of mortality in bacteremia.

2.4.2 Setting of bacteremia

Several studies have assessed the difference in mortality between CA and HA SAB. Lyytikäinen et al studied D28 mortality in SAB in Finland in 1995-2001. Mortality was 22% in HA and 13% in CA bacteremias ($p<0.001$) [81]. Another Finnish study found no association with HA or CA and D28 mortality [97]. An Australian study reported no difference in all-cause 7- and 30-day mortality rates between HA and CA-MRSA bacteremias but 1-year mortality was higher in the HA-MRSA bacteremias (48.3% vs 21.1%, $p = 0.023$) [102]. One year mortality probably reflects other prognostic factors of hospitalized patients and is not directly related to previous bacteremia. Most of the evidence suggest that there is no significant difference in mortality between HA and CA SAB [91,97,103-105].

2.4.3 Virulence factors

S. aureus harbours a large number of virulence factors such as surface proteins, enzymes and toxins that contribute to severity of disease and bacterium´s ability to cause invasive disease [106]. Peptidoglucans of the cell wall may have endotoxin-like activity, stimulating the release of cytokines by macrophages, activation of complement, and aggregation of platelets. Enzymes, such as protease, lipase, and hyaluronidase destroy tissue and may thus facilitate the spread of infection to adjoining tissues. Adhesins, such as fibronecting binding proteins, facilitate the adhesion of bactermium to host surface [20]. Production of exfoliative toxins is associated with staphylococcal scaled skin syndrome. Enterotoxins and toxic shock syndrome toxins are associated with food poisonings and toxic shock syndrome [107]. Among the most intensively studied virulence factor is Panton-Valentine leukocidin (PVL), a pore-forming toxin targeting polymorphonuclear leukocytes. It is thought to be linked to the pathogenesis of necrotizing pneumonia and necrotizing skin infections, although its role has recently been questioned [108-110]. PVL is frequently reported to associate with CA-MRSA infections [109,111]. However, according to the position statement from the International Society of Chemotherapy, prevalence reports suffer from a selective reporting bias towards CA-MRSA [112]. The prevalence of PVL in MSSA and MRSA is highly variable worldwide and estimations of PVL prevalence in *S. aureus* range from less than 2 % of strains in certain areas to more than 50 % in others [113].

Different sets of virulence genes are expressed during different phases of infection. During the initial stages the synthesis of surface proteins favours

successful colonization of host tissue, while in later phase exoproteins expression facilitates the spread to adjacent tissues [20]. Some virulence determinants are tough to designate to certain clonal lineages [17,106]. Virulence genes that are carried in the core variable genome are more strictly linked to certain *S. aureus* strains than virulence genes carried in the mobile genetic element of *S. aureus* [17].

S. aureus surface protein A (spa) that is also used for strain typing may account to the bacterium´s virulence properties. Spa is known to have antiphagocytic features due to its ability to bind the Fc portion of immunoglobulins [20]. This ability may dampen the opsonisation-dependent activation of complement. Spa is also known to bind thrombocytes providing a mechanism for bacterial cell adhesion to sites of vascular injury and thrombosis [114]. Taking these into account, it is possible that spa type has an impact on the outcome or clinical characteristics of SAB. Aamot et al found an association between spaCC 021 and all-cause hospital mortality [115].

2.4.4 Empirical therapy in *Staphylococcus aureus* bacteremia and the outcome

In a study by Kaech et al neither timing nor adequacy of antimicrobial therapy improved the outcome of SAB. Adequacy was defined as therapy with at least one antibiotic to which the microorganism was susceptible, given by an appropriate route and in an appropriate dosage for a sufficient period of time [95]. Conversely, one study reported a 1.7-fold increase in mortality when onset of proper antibiotic therapy was delayed over the breakpoint of 44.7 hours [116]. In this study, 62% of cases were MRSA bacteremias. In a small Swedish study, no diffirence in the outcome of invasive *S. aureus* infection was found between patients who received antimicrobial therapy within 24 hours or later (20% vs 17%, OR 1.24, CI 0.33–4.63) [91]. Paul et al have published a retrospective cohort study and meta-analysis including only HA-MRSA bacteremias. In the cohort study 67% received inappropriate empiric therapy and it was associated with poorer prognoses. Also in the meta-analysis, appropriate antimicrobial therapy had better prognostic impact (OR 1.98, 95% CI 1.62-2.44) [117]. Inadequate empiric therapy is significantly more frequent with MRSA than with MSSA bacteraemia (48 vs 12%, respectively) [118].

MSSA bacteremias are usually treated with second generation cephalosporins or anti-staphylococcal penicillins. Anti-staphylococcal penicillin appears to be superior to cephalosporins in treatment of MSSA bacteremia in some studies [119] but this was not confirmed in meta-analysis [120]. Finnish authors found corresponding results and discussed that the superiority of anti-staphylococcal penicillin might be

faded if the treatment is otherwise optimized by infectious diseases (ID-) specialist [121]. A Finnish prospective study showed that case fatality at three months in SAB patients with deep focus was lower if rifampisin was added to the standard treatment (17% vs. 38%, $p<0.001$). However, patients who did not receive rifampisin had more commonly other factors associated with higher mortality [122]. .A recent randomised, double-blind and placebo-controlled trial showed no overall benefit of adjunctive rifampicin over standard antibiotic therapy of SAB [123].

PSSA bacteremias could be treated with penicillin but it is not always an option since many laboratories have discontinued the testing of penicillin susceptibility [124]. Differences in the outcome of PSSA bacteremia treated with either penicillin or staphylococcal penicillin was addressed in a study by Nissen et al. In that retrospective study, 19% of PSSA patients treated with penicillin and 11% on patients treated with dicloxacillin died, but the difference was not statistically significant ($p=0.490$) [125].

Vancomycin is the most commonly used antimicrobial to treat MRSA bacteremia. Its nephrotoxicity makes achieving optimal plasma concentrations difficult and may lead to treatment failure. This may contribute to higher mortality associated with MRSA bacteremias. In countries where MRSA strains predominate in SAB, vancomycin is often used in empiric treatment of sepsis. This may impair the outcome of MSSA bacteremia. Also, newer antimicrobials are available for MRSA bacteremia but so far the abundance of literature favouring their use is not sufficient to replace vancomycin [126].

MSSA is usually susceptible to several antibiotic classes and receiving an inappropriate empiric antimicrobial therapy is uncommon. Thus, the outcome of MSSA bacteremia is mainly determined by other factors than initial treatment, such as underlying conditions. In MRSA bacteremias the impact of inappropriate antimicrobial therapy on outcome is a significant issue.

2.4.5 Impact of Infectious disease specialist's consultation

Infectious disease specialist´s consultation is shown to improve the outcome of SAB and bedside consultation is superior to telephone consultation [98]. Consultation may lead to more elaborate search of infectious focus and thus recognition of patients in need of longer treatment or surgical interventions. However, in one study including mainly MSSA bacteremias the benefit of consultation on outcome was significant only in univariate analysis [95].

2.5 Infection control

Most evidence on the effects of hospital hygiene interventions in the prevention of HA infections or the spreading of MRSA in health care settings is from observational studies and supports continuous use of combined interventions. High-quality evidence, based on randomized, controlled studies assessing the effect of infection control interventions is insufficient [127]. Since interventions are usually established as bundles, the effect of a single intervention is difficult or impossible to assess [128]. It has also been suggested that the effectiveness of MRSA control policies differ by ward speciality and that comparative studies across different health care contexts may not be feasible [129]. Many studies assess the effect of interventions in an ICU setting, although LTF are probably a significant reservoir of transmission [127,130-134]. The outcome in studies analysing the effect of control strategies may vary from incidence of specific infections to the rate of MRSA transmissions.

2.5.1 Screening, isolation and decolonization

Recognition of sources of MRSA transmission is the cornerstone of MRSA control. Screening of patients detects symptomless MRSA carriers as only 15% of MRSA carriers would be detected if microbiological cultures were performed on clinical basis only [135]. Screening is subsequently combined with isolation and/or decolonization of colonized patients. Conventional culture is the cheapest screening method, but takes three or more days to give a result. Chromogenic agars provide results in one or two days. PCR test is the fastest option as it gives a result in a few hours. It may be more sensitive but also more expensive than the other methods. Screening swaps are usually taken from nostrils and throat and wounds, sometimes also groins and perineum. Targeted screening means that only patients considered to be at high risk are screened. Universal screening means that all the patients are screened at admission to hospital [132].

A study from Scotland concluded that screening of all admissions to hospital with isolation of those identified as potential carriers proved most effective at reducing MRSA prevalence and most cost effective compared with no screening and targeted screening [136]. Universal screening also led to a decrease in MRSA bacteremias [137].

Cost-effectiveness of different strategies of screening and isolation were studied by Robotham et al [132]. Universal pre-emptive isolation and isolation of MRSA

positive patients identified using universal PCR screening was shown to lead to the greatest reduction in MRSA transmissions and infections when compared to the strategy of isolating and screening only high risk patients. However, this strategy is associated with inappropriate isolation and greatest costs and is probably cost effective only in high prevalence setting, where there is over ten percent MRSA positive on admission. Another review concluded that current evidence does not support replacing culture-based surveillance with rapid tests in settings where culture based surveillance is already implemented [138]. Authors also concluded that screening is likely to help decrease infection rates in hospitals but it cannot be firmly recommended as two randomised controlled trials included in the review failed to show its efficacy in an ICU setting [130,131]. Choosing between screening and isolation strategies requires consideration of local resources and MRSA prevalence.

Decolonization reduces the risk of MRSA or MSSA infection of carriers but its contribution to MRSA cross-transmission limitation is probably small [139]. In most studies, mupirosin nasal ointment is used with or without chlorhexidine soap. The effect of decolonisation therapy on occurrence of MSSA or MRSA infections has been studied extensively, also in RCTs [140,141]. The best evidence supporting the use of decolonisation is from patients undergoing orthopaedic or cardiothoracic surgery but preventive effects have also been documented from hemodialysis units, ICUs and gastrosurgery units [138,142-146]. Universal decolonization with clorhexidine bathing and nasal mupirocin is more effective than targeted decolonization or screening and isolation in reducing HA bacteremias due to any pathogen and positive MRSA clinical cultures in an ICU setting [147]. The baseline MRSA prevalence was not reported in the study, but it was probably high as the study was performed in the USA. Universal decolonisation strategy carries a risk of the emergence of resistance to decolonising agents [148]. Decolonisation is thus recommended only for selected patient groups for a limited time. Several studies have shown the efficacy of clorhexidin bathing in reduction of HA infections and transmissions of drug resistant organisms in an ICU setting and should be considered in this patient group [148-150].

Most studies observing the influence of isolation on MRSA transmission and infections have shown benefical effects [151,152] but some studies have shown no effect [153]. Some have suggested that barrier precautions may increase transmission if hand hygiene is consequently neglected [154]. Köck et al concluded in their review that where facilities for the isolation of MRSA patients are available, their use is recommended [138]. There are some studies that support the use of

pre-emptive isolation of all patients at admission [155-157]. This strategy is associated with high costs.

In conclusion, screening strategies are based on local epidemiology. Universal screening instead of targeted risk group screening may be justified in high-prevalence, endemic settings. Culture-based methods remain the golden standard in screening. Decolonization therapy is recommended for selected patient groups.

2.5.2 Hand hygiene

Hand hygiene has been shown to be a major element in preventing HA infections [158-160]. There are three commonly reported methods of measuring hand hygiene compliance: direct observation of practice, self-report of healthcare workers and indirect calculation based on hand rub product usage [161]. Direct observation is thought to be the most valid method and the generally accepted metric is the number of hand hygiene episodes/number of hand hygiene opportunities [161].

Sadsad et al conducted a mathematical modelling study to evaluate effect of screening, isolation, additional staff, cohorting and hand hygiene compliance of the staff on MRSA control [129]. They found hand hygiene compliance to be more successful in reducing MRSA rates than any other control measure regardless of ward speciality. The largest decrease in the average daily MRSA prevalence was noted when hand hygiene compliance increased form level of 35% to 55-60%. In an increase above this level, no such significant change in MRSA findings was noted. Also, another study has suggested that only an improvement in hand hygiene from a very poor level has any significant impact on *S. aureus* ward-level prevalence and colonized patient-days, whereas improvement above levels of 40% compliance has only a small effect [162]. Use of gloves in the care of patients does not dilute the importance of hand rub use in all care [163].

2.5.3 Institutional issues

The backbone of succesful infection control is understanding that it is the responsibility of everyone involved in the care of patients and thus an everyday component of patient care [128]. Implementation of infection control strategies may require a change in the institutional culture and overcoming the initial resistance of change may be challenging. Welsh et al studied factors of institutional culture in 33 hospitals where implementation of infection prevention interventions

has been succesful. They found the frontline dedication of staff and administration and collaboration to be vital. The administration should accept accountability, provide tools, supplies and funds for the infection control work and show their own commitment. Frontline staff should provide ideas and expertise, since they understand the best, what must be changed in their units. Re-education of the staff and repetitive unit-specific feedback were also essential for success [164]. A successful infection control program requires support of the hospital administrator as they provide the resources. In a report by O´boyle et al, lack of adequate resources was the most common reason for non-performance of essential infection control tasks [165].

2.5.4 Antimicrobial stewardship programs

Strategies to restrict unnecessary antibiotic use are an important measure to limit the dissemination of MRSA by reducing selection pressure. Especially exposure to fluoroquinolones is associated with an increased risk of MRSA acquisition [166]. Antimicrobial stewardship programs are shown to decrease the total antimicrobial consumption, use of broad-spectrum antibiotics and costs without causing significant adverse effects such as excess deaths [167]. Programs may contain such objectives as the use of empiric therapy according to guidelines, properly timed de-escalation of therapy, switching the route of administration from intravenous to oral and infectious disease specialist consultations. Decrese in the percentage of methicillin-resistant strains amongst *S. aureus* findings from 47% to 30% after iniation of intervention program to optimize antibiotic use was reported in Argentina [168]. A pooled analysis of 17 studies showed 37% reduction in the incidence of MRSA infections or colonizations after implementation of antibiotic stewardship. In several studies, antibiotic stewardship programs were implemented alongside with with other infection control measures [169].

2.5.5 Environmental cleaning

The role of environmental cleaning and decontamination in MRSA control is undoubtedly significant, but the quantity of their effect is unclear, as they are not usually evaluated against patient outcome. Environmental cleaning is also seldomly studied as a single intervention but as a part of other hospital hygienic interventions.

Microbes shed by patients and staff contaminate the health care environment and MRSA may persist on the surfaces for days, which increases the risk of onward transmission [22]. There are reports from wards with on-going MRSA transmission where the reservoir of transmissions was contaminated environment [170,171]. Awareness of the importance of basic hospital cleaning in MRSA control has risen during recent decades [172] but there are still many controversial issues regarding cleaning techniques, monitoring and standards for surface cleanliness as well as the role of cleaning in controlling HA infections [173].

3 AIMS OF THE STUDY

The aims of the present study were:

1. To study the epidemiology of HA-MRSA and potential influence of improved hospital hygiene (study I)

2. To compare the clinical characteristics and outcome of MRSA and MSSA bacteremia and factors associated with the outcome of bacteremia (study II)

3. To study the possible association of *S. aureus* spa type on clinical characteristics of bacteremia (study III)

4. To compare spa type distribution of invasive MRSA and MSSA strains (study III)

5. To study the epidemiology of MRSA, PRSA and PSSA bacteremia in population-based setting and possible influence of improved hospital hygiene (study IV)

4 MATERIALS AND METHODS

4.1 The setting

The study was performed in Pirkanmaa county with a population of 522 993 at the beginning of the study in the year 2001. Tertiary care is provided by Tampere University Hospital (TAUH) and secondary care by three, from the year 2015 two, Disctrict Hospitals and Tampere City Hospital (secondary care hospitals, SH). During the study period, primary care was provided by 18-20 health care centres. There are also approximately 200 institutions for long-term care in the county. For study I, cases from health centre wards and long-term units were combined since many health centre wards also offer long-term care (LTF).

The city of Jämsä joined the Pirkanmaa Health District in 2013. The population and cases from Jämsä were excluded from study IV in the analyses of the incidence of SAB.

4.2 Case definition

In study I, a case was defined as a person with a new finding of MRSA cultured from either a screening or a clinical sample during August 2001 to December 2014.

In studies II-IV, a case was defined as a patient with at least one blood culture positive for *S. aureus* associated with the signs and symptoms of an infection. In study IV, if a single patient had repeated bacteremias, each bacteremic episode was regarded as a distinct case if the interval between episodes was three months or more. In study II, bacteremia episodes were regarded as distinct episodes if they were diagnosed at least 30 days apart.

4.3 Data sources

Several sources were used to collect the data for the studies. MRSA database was used in studies I and III. The database is run by the Pirkanmaa Hospital District´s (HD) Infectious Disease Unit (IU) and it includes all diagnosed MRSA carriers in Pirkanmaa. MRSA can be recovered either from clinical samples or screening samples. Only the first positive sample of each carrier is recorded in the database. It contains data on demographics, the type of acquisition (HA, CA or findings in healthcare workers (HCW)), the type of sample (clinical or screening), the site of transmission of HA-MRSA (i.e. TAUH, SH, LTF and abroad) and strain type.

SIRO is a Finnish Hospital Infection Program. SIRO criteria were used to define the health care association of bacteremia in studies II-IV. SIRO criteria are based on CDC´s definition [14]. SIRO database is held by THL and TAUH reports HA bacteremias to SIRO four times per year. SIRO database was used in study IV to classify bacteremia´s HA.

NIDR at the THL collects notifications of positive blood cultures from all laboratories in Finland. The date, identity code, age, gender and information on the methicillin resistance of *S. aureus* isolates are recorded in the NIDR. Data from NIDR was used to study the *S. aureus* incidence in Pirkanmaa. Public NIDR data from other parts of Finland was also used for comparison (study IV).

SAI is a commercial surveillance system for antibiotics and infections. Its purpose is to enable local surveillance of infections and antibiotic resistance. Data on MRSA bacteremia episodes for studies II and IV was retrieved from SAI. For each MRSA case three gender- and age-matched controls with MSSA bacteremia were selected. Data on MSSA bacteremia episodes was obtained from the central laboratory of Pirkanmaa county, Fimlab.

HILMO is a national hospital discharge database held by THL. It contains data on patients hospital admission and discharge. HILMO data was used in study IV to classify SABs HA or CA.

Overview of the study designs is presented in Table 1.

Table 1. Overview of the study desing

Study	I	II	III	IV
Data source	Pirkanmaa MRSA database	SAI and hospital records	SAI and hospital records	NIDR, SIRO, HILMO, hospital records
Study design	Population-based, retrospective	Cohort study, case-control study, retrospective	Cohort study, retrospective	Population based, retrospective
Included cases	All newly recognized HA-MRSA carriers	MRSA bacteremia patients reported to SAI and their MSSA controls	MRSA bacteremia patients reported to SAI and their MSSA controls	All SAB patients reported to NIDR
Study period	8/2001-12/2014	2002-2013	2004-2013	2005-2015
Number of cases or episodes	3527	504	462	1527
Recurrent cases	No	Episodes were considered separate if noticed at least 30 days apart.	No	Episodes were considered separate if noticed at least three months apart

HA= health care associated, HILMO=National Hospital Discharge Database, MRSA=Methicillin resistant *Staphylococcus aureus,* MSSA=Methicillin susceptible *Staphylococcus aureus*, NIDR=National Infectious Diseases Register, SAB=*Staphylococcus aureus* bacteremia, SAI=Commercial surveillance system for antibiotics and infections, SIRO=Finnish National Surveillance Program of Blood Stream Infections

4.4 Data on site and type of MRSA admission

Study I

The MRSA database contains data on the site and type of MRSA transmission. The type and the site of transmission are evaluated by two experienced infection control (IC-) nurses by studying the patient history [5]. The definition of the type of transmission changed during the study period:

1) From 2001 to 2005, MRSA transmission was defined as HA if the case had a history of in-patient admission after September 2001 in any healthcare facility (TAUH, SH or LTF) in Pirkanmaa.

2) From the year 2006 onwards all cases who had been treated in any healthcare facility in Pirkanmaa during the previous 2 years were considered HA cases.

3) If the case did not fulfil these HA criteria and was not a HCW, the transmission was considered to be CA.

The evaluation of the site of HA transmission (TAUH, SH or LTF) was based on the following criteria

1) If MRSA was found in a clinical sample taken within 48 h after the hospitalization, the transmission was linked to the former in-patient hospitalization (from the year 2006 only two preceding years were counted).

2) If screening samples were negative on hospital admission but samples (clinical or screening) taken 48 h or later after the hospitalization were found to be positive, the transmission was linked to the current hospitalization.

4.5 Definition of health care associated bacteremia

Studies II-IV

SIRO criteria used in studies II-IV defines HA bacteremia as follows: infection was defined HA if the symptoms started more than 48h after hospital admission or the bacteremia was related to surgical operation within the past 30 days or other invasive procedure within the last 10 days. Patients on chronic renal replacement therapy (hemo- or peritoneal dialysis) or neutropenic due to treatment of malignancy were classified as having a HA bacteremia [12,14]. For studies II and III the data on bacteremias HA was obtained by the review of individual patient charts by the investigator. In study IV also SIRO and HILMO data was used.

4.6 Data on clinical characteristics of bacteremia

Studies II-III

Data on clinical characteristics of SAB for studies II and III was collected from the hospital records. Partially the same material was used in an earlier publication covering the years 2002-2010. For this study, the material was expanded to also cover years 2011-2013 [174]. A data collection form (see appendix) was used. Table 2 shows the variables whose association with the risk of MRSA bacteremia or D28 mortality was analysed in study II. Data on the focus of bacteremia was used also in study III to analyse its association with spaCC.

Table 2. Variables included in study II to analyse the factors associated with the risk of MRSA bacteremia or mortality in SAB within 28 days

Underlying condition	Body mass index
	Current smoking
	Cardiac disease
	Chronic kidney disease
	Chronic or traumatic ulcer
	Dermatitis
	Diabetes (type 1 or 2)
	Liver cirrhosis
	Foreign body[1]
	Chronic renal replacement therapy
	Glucocorticoid therapy ≥5 mg/day (within previous month)
	Immunosuppressive therapy
	Prior surgical operation
	Other invasive procedure
	Health care associated bacteremia
Focus	Pneumonia
	Skin or soft tissue infection
	Deep abscess
	Native arthritis
	Central venous catheter
	Infected foreign body[1]
	Endocarditis
	Spodyodiscitis or osteomyelitis
	Mediastinitis
	Unknown focus
	Echocardiogram performed
Treatment	Empiric antibiotic effective
	Treatment in intensive care unit
	Acute renal replacement therapy
	Invasive ventilation

[1]Articular prosthesis, vascular prosthesis, pacemakers or osteosynthesis material

MRSA=Methicillin resistant *Staphylococcus aureus*, SAB=*Staphylococcus aureus* bacteremia

4.7 Microbiological methods

MRSA screening samples, clinical samples and blood cultures were collected and processed from the patients on clinical basis and retrospectively analysed in our study. Spa typing of bacteremic MSSA and those MRSA strains that had not been typed earlier was performed solely for this scientific purpose.

4.7.1 Cultures

The central laboratory, Fimlab laboratories, analyse all microbiological samples in the county. MRSA screening samples were inoculated onto oxacillin resistance screening agar (ORSAB, OXOID) and incubated for 48 h at 37 ºC until 2008. Since 2008, specimens have been inoculated onto chromogenic agar (CHROMagar MRSA) and incubated for 24 h at 37 ºC. *S. aureus* was confirmed using coagulase test.

The blood culturing instrument used until 2007 was BACTEC 9240 (BD Diagnostic Systems, Sparks, MD, USA). Since then, BacT/ALERT 3D (bioMerieux SA, Marcy L'Etoile, France) has been used. Data on the number of blood cultures taken in the county was obtained from Fimlab laboratories (study IV).

4.7.2 Detection of methcillin resistance and penicillinase production

Methicillin resistance was confirmed using Clinical and Laboratory Standards Institute (CLSI) guidelines until 2011 and since then using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [175]. MRSA strains were confirmed by determining the oxacillin minimum inhibitory concentration using the Etest, and testing for the presence of the *mecA* gene. *MecA* testing was performed at the THL until 2005 and thereafter at Fimlab using GenoType MRSA (Hain Lifescience). Penicillinase production was detected using a disk diffusion test and a cloverleaf test.

4.7.3 Strain typing and spa clonal complexes

Spa typing has been used as a primary typing method since 2009. Until then, PFGE was used. Spa typing is performed at THL using Ridom StaphType program (Ridom GmbH, Würzburg, Germany) [63]. Strain typing is performed each time a

new MRSA carrier is found or MRSA is recovered from a blood culture. The most commonly found spa type (t067) in Pirkanmaa is shown to have 97% concordance with PFGE strain type FIN-16 [5,63]. This strain (FIN-16/t067) was the designated Pirkanmaa epidemic strain (PE-MRSA) for study I. For study III, bacteremic MSSA control strains and those MRSA strains that had not been spa typed before year 2009 were spa typed.

Spa types were grouped into spaCCs using the Based Upon Repeat Pattern (BURP) algorithm of the Ridom StaphType software (version 2.1.1), which supports the identification of epidemiologically related strains [65,176]. The default setting of the algorithm was used: spa types shorter than 5 repeats were excluded and spa types were clustered into the same spaCC if costs distance was less than 6.

Spa type data was used to identify MRSA transmissions and bacteremias related to Pirkanmaa MRSA epidemic (study I and IV), to compare strain distribution in MRSA and MSSA bacteremias and to seek potential association of spa type and spaCC with clinical characteristics of *S. aureus* bacteremia (study III).

4.8 Hospital hygiene interventions in Pirkanmaa

To combat the MRSA epidemic, several hospital hygiene interventions were launched stepwise during the study period in TAUH, LTF and other health care institutions in Pirkanmaa county. Summary of infection control interventions (other than screening) is presented in Table 3. The control policy is based on Finnish national guidelines, published by THL in 1995 and updated in 2004 and 2014 [177]. These guidelines give instructions for care of MRSA carriers and MRSA exposed patients and recommend active screening of risk groups.

Table 3. Summary of infection control interventions (other than screening) launched during the methicillin-resistant *Staphylococcus aureus* (MRSA) epidemic in Tampere University Hospital (TAUH), long-term facilities (LTF) and all heath care institutions in Pirkanmaa county.

Emphasizing the use of standard precautions in all care	Throughout the epidemic
Emphasizing the use of contact precautions in care of MRSA carriers in hospitals	Throughout the epidemic
MRSA cohort wards in Tampere city hospital and in some health care centres.	2002-2004
Infection control promotion project in LTF	2004
Link nurse network established in LTF and primary care centres	2004-
MRSA cohort ward for surgical patients in TAUH	2008-
MRSA dialysis in TAUH	2009-
Infection control promotion project in TAUH	2009
Three mobile infection control nurses were recruited to visit LTF and other health care institutions regularly	2010-
Working group for MRSA epidemic was established in TAUH	2011-
Reinforcement of link nurse network in TAUH	2012-
MRSA decolonization for selected patient groups in TAUH	2012-
Regional on-line handbook to guide the correct choice of empiric antimicrobial therapy was published	2014

4.8.1 Standard and contact precautions, compliance promotion and resources

Adherence to standard precautions in all care was emphasized throughout the epidemic. The implementation of contact precautions was required in the care of all MRSA carriers. This means treatment in a single room and the use of gowns, gloves and masks when in close contact with a patient. To promote infection control, a link nurse network was established in primary care centres and LTF in 2004 as part of interim hospital hygiene promotion project (MARS matkaan!). The projects sought to harmonize infection control policies in different units and to strengthen cooperation between regional operators and IU.

As the number of MRSA carriers increased it became difficult to arrange contact isolation rooms for all MRSA patients. Cohort units for MRSA patients were set up in 2002-2004 in Tampere City Hospitals and in some health centres. In TAUH, MRSA cohorts were set up in 2008 in the gastroenterology unit and in 2009 for patients in chronic renal replacement therapy.

Adherence to standard and contact precautions was additionally promoted by another interim project in 2009 in TAUH. During the project, staff´s hand hygiene compliance was directly observed by IC- and link nurses. The project revealed that the level of compliance was generally poor (16%) in the beginning of the project but improved up to 50% during the project.

Two mobile IC-nurses, based in IU, were recruited in 2010 to visit primary care centres and LTF regularly to educate personnel on infection control. The link nurse network was later expanded to secondary care hospitals and reinforced in TAUH in 2012. Their job description was defined more precisely in 2012. Link nurses monitor the transmission rates and observe hand hygiene compliance in their wards and analyse feedback reports with ward personnel. In TAUH from the year 2012 on it was recommended that link nurses could use one day per week for infection control work on somatic wards and one day per every three weeks on other wards. Two additional infectious disesases specialists and IC-nurses were recruited in TAUH in 2013. Nine IC-nurses and five ID-specialists were given a specific area of responsibility, three of the nurses and one ID-specialist work mainly in Pirkanmaa HD area (mobile work). ID-specialist and IC-nurse are together responsible of hospital hygiene promotion and surveillance of

transmission and infection rates in their area. Smaller campaigns and competitions to promote hand hygiene are organized annually.

Hand-rub use in TAUH wards is monitored continuously. To our study I, hand-rub consumption was assessed using the amount of hand-rub ordered (in litres) in each ward in TAUH, divided by 1000 patient days.

4.8.2 MRSA screening policy in Pirkanmaa

Screening policy was expanded twice during the study period (Screening period I, II and III). Screening practises are presented in Table 4. Screening samples were taken from throat, nostrils, catheters, drainage tube sites and skin lesions. HCWs were screened only if they fulfilled other screening criteria. Universal screening has also been recommended to primary care centres and LTF since 2011. Only mothers in labour without previous hospitalization and children are excluded.

Table 4. MRSA Screening recommendations

	Time span	Patients
Screening period I	2001-5/2008	Patient admitted to TAUH if they had been treated in specified institutions with known existence of MRSA
Screening period II	6/2008-9/2011	Patients admitted to TAUH with a history of in-patient care in any healthcare facility in the county after 2001
Screening period III	10/2011-	All patients admitted to any hospital in Pirkanmaa except children and women in labour

MRSA= methicillin-resistant *Staphylococcus aureus,* TAUH= Tampere University Hospital

4.8.3 Commitment of the board of Health District and all health care institutions

At the end of 2011, a MRSA Working Group was established by the Board of Pirkanmaa HD. Its duty is to supervise the actions and surveillance in the epidemic. The members of the MRSA Working Group are the medical director, heads of hospitals´ medical divisions, personnel manager of TAUH and three infectious disease specialists. The working group aims to identify healthcare units where urgent actions for improvement of infection control practices are needed and guide the use of financial resources allocated for infection control.

4.8.4 Feedback

A summary of the current MRSA transmission rate and site is reported monthly, later on 4 times per year, to the MRSA Working Group. The report is also distributed to the medical directors and head nurses in LTF, health centres and SHs in the county. The report is given to the chief medical administrator and board of health district three times per year. Since the beginning of MRSA surveillance, IC-nurses have analysed each new MRSA transmission with the ward personnel. HaiPro reports are also used to analyse the tranmissions on ward level.

4.8.5 MRSA eradication and decolonization

According to Finnish and regional guidelines, MRSA eradication therapy containing topical disinfection treatment and nasal mupirocin ointment therapy combined with antimicrobial treatment is offered for HCWs, who have been shown to carry MRSA and for patients with recurrent skin and soft tissue abscesses. Those children who have only relative indication for eradication therapy do not receive systemic antibiotics.

From 2012 to 2013, MRSA decolonisation therapy containing topical disinfection treatment and nasal mupirocin ointment was given to MRSA carriers who were treated in the ICU or when there were no single rooms available in a regular ward. Decolonization therapy was also given during their hospital stay to MRSA carriers who had secreting wounds or exfoliating dermatitis. Since 2013, it has been recommended to all MRSA carriers during their hospital stay.

4.8.6 Antibiotic use

IU counsels the antibiotic use in health care units and in hospitals in the HD. A regional on-line handbook to guide the correct choice of empiric antimicrobial therapy in infections treated in hospital has been available since 2014. IU has paid attention to rational use of antibiotics as a part of HA infection prevention. Special attention has been paid to avoiding unnecessary use of fluoroquinolones.

4.9 Statistical methods

A SPSS package (version 22, IBM Corp., Armonk, NY) and STATA (version 13.0, StataCorp, TX) were used for statistical analyses. All the studies had categorical data, which was analysed using Chi Square test or Fisher´s test. In study II conditional logistic regression was used for analysis of underlying diseases and foci and logistic regression was used for analysis of variables associated with D28 mortality. Results are presented in OR with 95% CI.

4.10 Ethical considerations

The study was approved by the Ethical Review board of the Pirkanmaa Health District. Informed consent was not required because all studies I-IV were of retrospective design based only on the review of hospital patient record data.

5 RESULTS

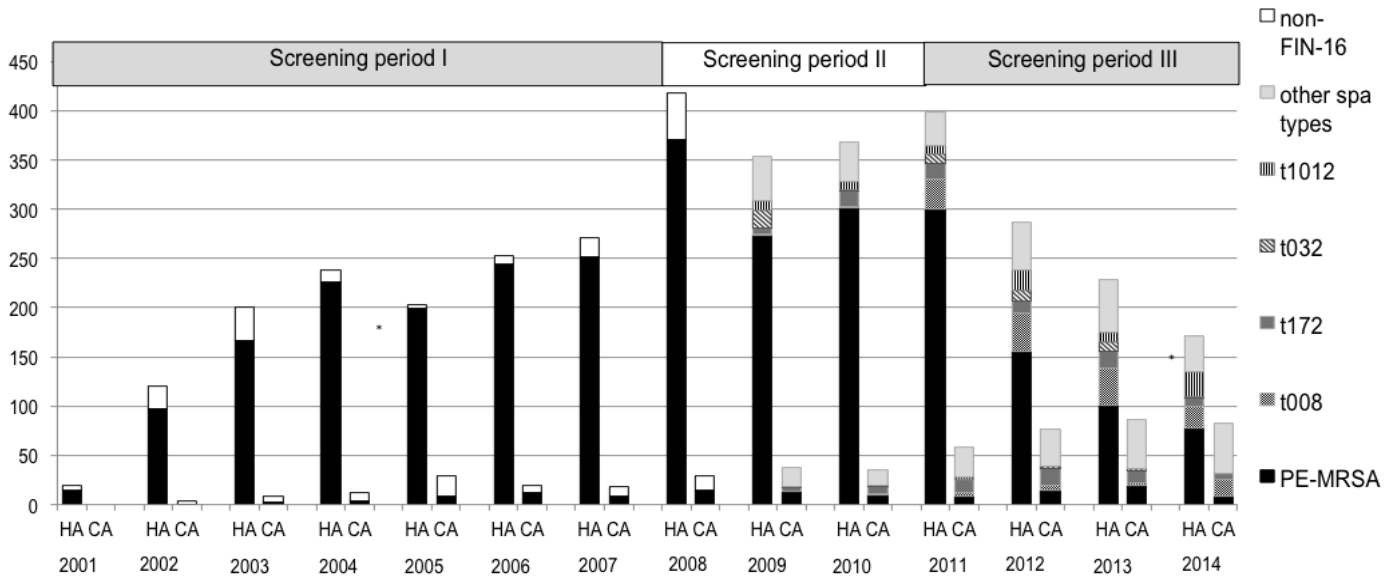
5.1 New MRSA findings in Pirkanmaa county (I)

During the years 2001-2014, a total of 4118 new MRSA cases were identified in Pirkanmaa, of which 3527 (86%) were classified as HA. There were 39 (1%) cases in HCWs and 53 cases (1%) were HA in other HDs in Finland. In all, 436 cases (11%) cases were CA and 62 (2%) cases were transmissions from abroad. In one case, the site of transmission was not defined.

Figure 1 presents the number of HA-MRSA and CA-MRSA in relation to the year of epidemic. At the beginning of the epidemic, HA-MRSA cases increased constantly reaching their top in 2008 when 418 cases were found. In the year 2009 the number of MRSA cases decreased temporarily, but started to rise again in 2010. Since 2011, HA-MRSA cases decreased constantly. The number of new HA-MRSA cases was 57% less in the year 2014 (n = 171) as compared with the year 2011 (n = 399).

The number of CA-MRSA cases increased slowly but constantly reaching its top in 2013 when 86 CA-MRSA cases were found.

Figure 1. MRSA strain type in relation to the type of acquisition of MRSA and different years within the epidemic. Boxes indicate the screening periods (I, II or III). The proportion of PE-MRSA among HA-MRSA cases decreased significantly from 98% (199/203) in 2005 to 45% (100/224) in 2013 ($p < 0.001$, Chi-square test).



HA= health care associated, CA= Comminuty-Aqcuired, MRSA=Methicillin-resistant *Staphylococcus aureus*, PE-MRSA= Pirkanmaa epidemic MRSA

5.2 Strain type distribution in MRSA cases (I)

Strain type distribution in MRSA cases is presented in Figure 1. Until January 2009 PFGE was used for strain distinction and 17 different FIN types (n = 1729) in HA-MRSA cases were found. Of cases, 91% (1574/1729) represented PE-MRSA (FIN-16).

Since the adoption of spa typing in January 2009, 98 different spa types have been found (n = 1798) in HA-MRSA, and 67% (1204/1798) of cases were of PE-MRSA (t067). Other most prevalent spa types were t008 (7%), t1012 (5%), t172 (4%) and t032 (3%).

During the whole epidemic, 79% (2778/3527) of all HA-MRSA cases have been PE-MRSA. Proportion of PE-MRSA in HA-MRSA cases has decreased from its peak 98% (199/203) in 2005 to 45% (100/224) in 2013 (p<0.001).

In CA-MRSA cases, PE-MRSA comprised 29% (126/436) of cases. The proportion of PE-MRSA cases has decreased from 63% (12/19) in year 2006 to 11% (9/83) in 2014 (p<0.001).

PE-MRSA is PVL-negative, but PVL production in not routinely tested in MRSA strains Pirkanmaa.

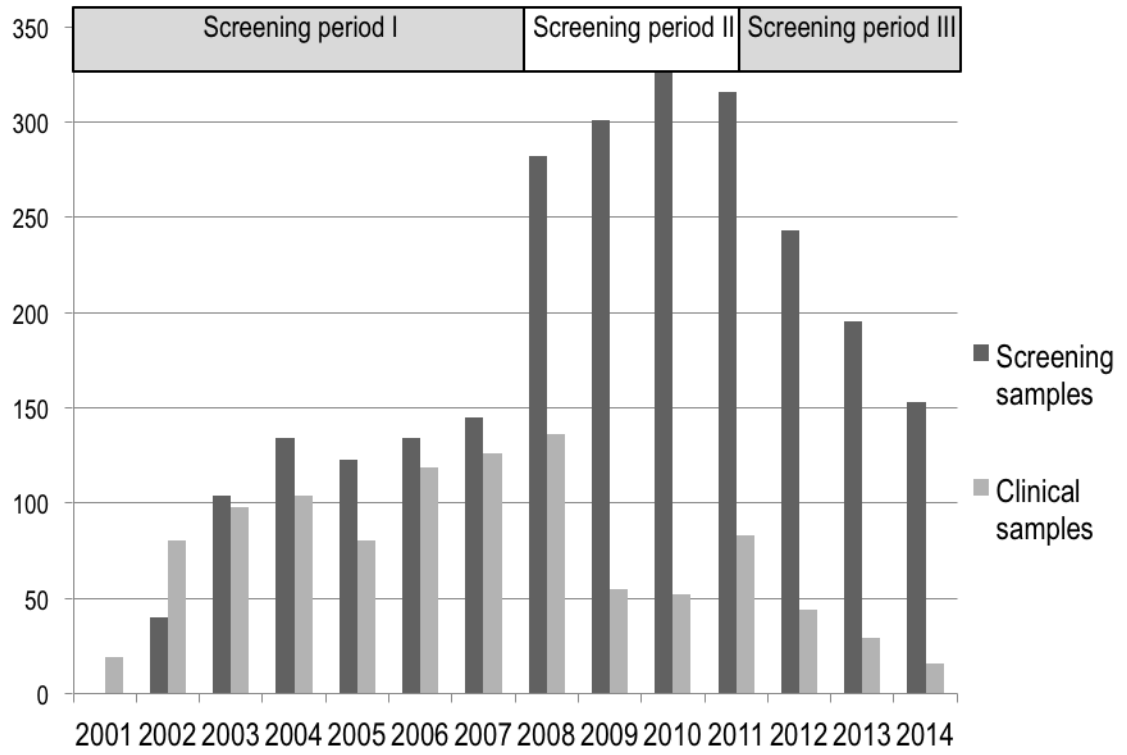
5.3 HA-MRSA findings in screening samples and in clinical samples (I)

Figure 2 presents the number of HA-MRSA cases found in MRSA screening samples and in clinical samples in relation to the year of epidemic and screening period. During the whole study period, screening samples comprised 71% (2493/3527) and clinical samples 29% (1034/3527) of HA-MRSA findings. During the first two years of the epidemic 71% (99/139) of new HA-MRSA cases were found from clinical samples. Since 2003 the majority of HA-MRSA cases were found in screening samples and in 2014 only 9% (16/171) were found in clinical samples. The proportion of cases found in clinical samples decreased in relation to screening periods. During screening period I, 46% (676/1469) of new cases were found in clinical samples. In screening periods II and III, the proportion decreased to 19% (238/1235) and 15% (120/823), respectively (p<0.001 for comparisons screening period I vs screening period II and screening period I vs screening period III).

Of the clinical samples, 71% (734/1034) were from superficial focus, 21% (216/1034) from urine samples, 3% (35/1034) from blood cultures, 3% (35/1034) cultures from other deep focus and 1% (14/1034) from undefined focus.

The percentage of PE-MRSA in clinical samples was 85% (884/1035) and 78% (1894/2439) in screening samples. The proportion of PE-MRSA in clinical samples has decreased from its peak of 99% (79/80) in 2005 to 45% (13/29) in 2013 ($p<0.001$). In screening samples the proportion of PE-MRSA decreased from 98% (120/123) in 2005 to 45% (69/155) in 2014 ($p<0.001$).

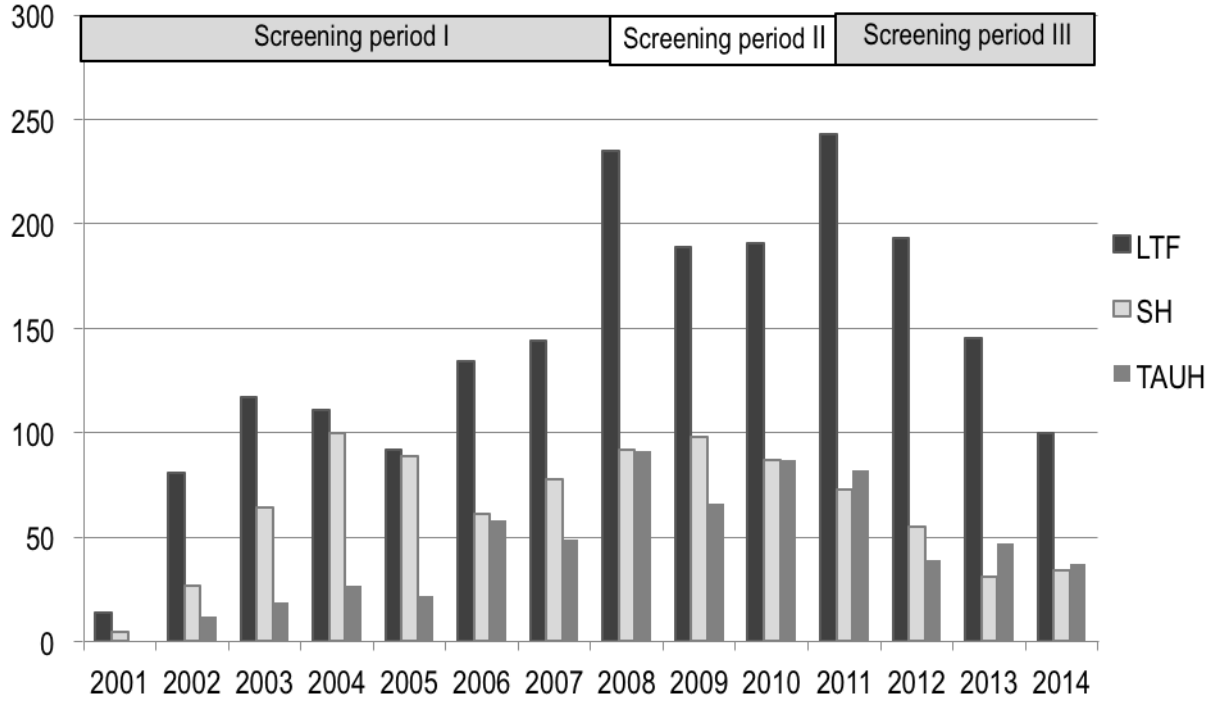
Figure 2. The number of health care associated methicillin-resistant *Staphylococcus aureus* cases recovered from clinical and screening samples in relation to year of transmission and screening period.



5.4 Site of transmission of HA-MRSA (I)

The site of MRSA transmission (LTF, SH or TAUH) in relation to the year of epidemic is presented in Figure 3. The majority of new HA-MRSA cases throughout the whole epidemic have been encountered in LTF (56%, 1989/3527). Findings in SH comprised 25% of cases (894/3527) and findings in TAUH comprised 18% (636/3527) of cases. In eight cases the site of HA-MRSA transmission was missing. Throughout screening period II the number of new cases remained high both in LTF and in hospitals (TAUH and SH). During screening period III the number of new cases started to decrease both in LTF and in hospitals.

Figure 3. The site of MRSA transmission in relation to the year of epidemic is presented in Figure 3.



MRSA=methicillin-resistant *Staphylococcus aureus*, LTF=long-term facilities, SH=secondary care hospital, TAUH=Tampere University Hospital

5.5 Hand-rub use and screening activity (I)

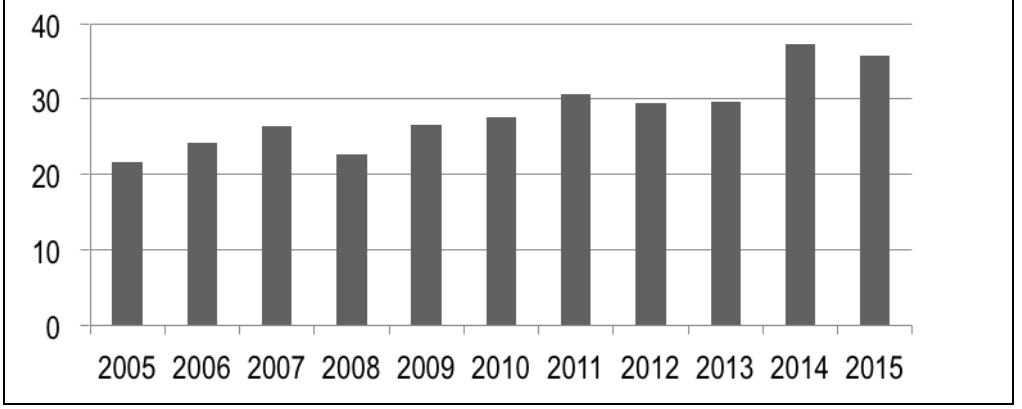
To evaluate the effectiveness of hand hygiene promotion in TAUH, the use of alcohol hand-rub from 2005 to 2014 was calculated using the amount of hand-rub ordered (litres) in each department in TAUH, divided by the number of patient days. The hand-rub use more than doubled from 42.0L/1000 patient days in 2005 to 108.0L/1000 patient days in 2014 (Original article I, Figure 3).

The number of screening samples taken each year was calculated. As the screening recommendations changed, the average number of screening samples increased. The number of screening samples taken yearly during screening periods I, II and III were approximately 48 000, 132 000 and 228 000, respectively. Since several screening samples are taken from each patient, the number of screened patients is actually much smaller.

5.6 Incidence of *Staphylococcus aureus* bacteremia (IV)

The total number of SAB cases in Pirkanmaa notified to NIDR from 2005 to 2015 was 1527 in 1423 patients. The annual incidence of SAB ranged from 21.6 (2005) to 37.2 (2014) per 100 000 population. The overall SAB incidence within the study period is presented in Figure 4.

Figure 4. The overall incidence of *Staphylococcus aureus* bacteremia (cases per 100 000 population) in Pirkanmaa from 2005 to 2015. Source: National Infectious Diseases Register.



The median age of cases was 67 years (range 0-100) and 61.8% (944/1527) were male. Of cases, 54.7% (835/1527) were HA. The incidence was highest in patients ≥75 years of age (117.4/100 000 population), in patients 65-74 years of age (61.4/100 000 population) and in those under 1 year of age (39.4/100 000 population). The lowest incidence was seen in patients 15-24 years of age, 6.0/100 000 population. The incidence was 35.7 per 100 000 in males and 21.2 per 100 000 in females.

5.7 Incidence of MRSA and MSSA bacteremia in Pirkanmaa and other parts of the country (IV)

Incidence of MRSA and MSSA bacteremia per 100 000 population in Pirkanmaa county and in other parts of the country is presented in the original article IV, Figure 1. The incidence of MSSA cases increased during the study period from 19.9 to 35.2 in Pirkanmaa and from 18.8 to 37.1 in other parts of the country. The incidence of MRSA cases increased in Pirkanmaa reaching its top in 2011 when the incidence was 4.5 per 100 000 population. Since then, the incidence has decreased to the average level of the country. In other parts of the country a slight increase was seen in the incidence of MRSA bacteremia cases.

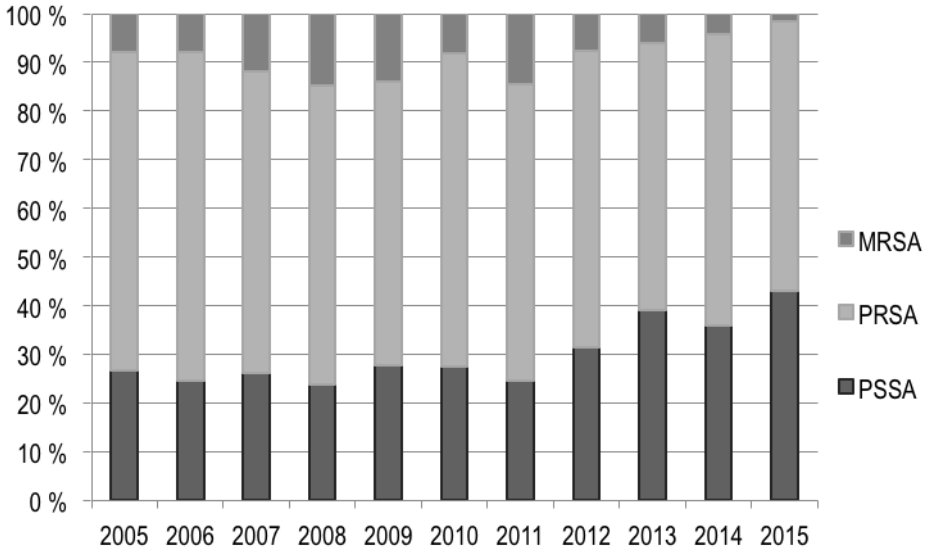
In Pirkanmaa, annual variance was observed in the incidence of both CA and HA SAB cases but an increasing trend in both groups was observed. The incidence of CA cases was lowest in 2008 (7.9/100 000 population) and highest in 2014 (18.5/100 000 population). The incidence of HA cases was lowest in 2005 (11.5/100 000 population) and highest in 2015 (20.8/100 000 population).

5.8 Proportions of PSSA, PRSA and MRSA in SAB (IV)

Proportions of PSSA, PRSA and MRSA in SAB in Pirkanmaa are presented in Figure 6. During the whole study period, most infections were caused by PRSA 60.5% (924/1527). MRSA caused 8.5% (130/1527) and PSSA 31.0 % (473/1527) of the cases. The proportion of PSSA increased from the lowest 23.9% (26/109) in 2008 to 43.1% (78/181) in 2015. The proportion of PRSA declined from 67.5% (77/114) in 2006 to 55.2% (100/181) in 2015. The proportion of MRSA was highest in the year 2008 14.7 % (16/109) and lowest in the year 2015 1.7% (3/181).

The proportion of PSSA in HA and CA bacteremias was similar. PSSA accounted for 31.4% (262/835) of HA cases and 30.5% (211/692) of CA cases. PRSA was more common in CA cases than in HA cases. It comprised 56.2% (469/835) of HA cases and 65.8% (455/692) of CA cases. MRSA accounted a higher proportion of HA cases (12.5%, 104/835), than of CA cases (3.8% 26/692).

Figure 6. Proportions of PSSA, PRSA and MRSA in Staphulococcus aureus bacteremia in Pirkanmaa in years 2005-2015



PSSA=Penicillin-susceptible *Staphylococcus aureus, PRSA*=Penicillin-resistant *Staphylococcus aureus*, MRSA=Methicillin-resistant *Staphylococcus aureus*

5.9 Number of blood cultures taken in the county (IV)

The number of blood cultures and the rate of *S. aureus* isolates per 10 000 blood cultures from 2005 to 2015 is presented in Table 5. The annual number of blood cultures in Pirkanmaa county increased from 25 801 in 2005 to 46 785 in 2015. The rate of *S. aureus* isolates per 10 000 blood cultures was 39.1 in 2005 and 38.7 in 2015. The average annual rate during the study period was 39.7/10 000 blood cultures.

Table 5. The number of blood cultures in Pirkanmaa county and the rate of Staphylococcus aureus (*S. aureus)* isolates per 10 000 blood cultures from 2005 to 2015

Year	Annual number of blood cultures	The rate of *S. aureus* isolates per 10 000 blood cultures
2005	25 801	39.1
2006	28 295	40.3
2007	29 055	43.4
2008	30 419	35.8
2009	31 361	41.1
2010	33 338	40.5
2011	36 205	41.7
2012	38 101	38.1
2013	40 016	37.0
2014	46 024	40.6
2015	46 785	38.7

5.10 Risk factors of MRSA bacteremia (II)

Analysis of the association of underlying conditions with the risk of MRSA bacteremia was performed for the variables presented in the materials and methods section. MRSA cases were more often smokers (OR 2.34, 95% CI 1.27-4.32), had previous surgery (OR 2.32, 95% CI 1.43-3.76), glucocorticoid therapy (OR 1.82,

95% CI 1.12-2.93) and were classified as having a HA bacteremia (OR 4.23, 95% CI 2.47-7.24) than MSSA controls. Smoking data was missing from 24% of cases. Similar risk factors were observed in comparison to PRSA controls. In comparison of MRSA cases to PSSA controls, only previous surgery (OR 2.46, 95% CI 1.29-4.69) and HA (OR 4.56, 95% CI 2.21-9.37) were significant risk factors. There was no significant difference in any underlying conditions between PRSA and PSSA bacteremias.

5.11 Focus of *Staphylococcus aureus* bacteremia (II)

The focus in MRSA, MSSA, PRSA and PSSA bacteremia is shown in Table 6. Focus data were missing from 4% (20/504) of cases. The most common focus in all the subgroups was SSTI. The second most common focus was central venous catheter (CVC) in MRSA, MSSA and PRSA bacteremias and pneumonia in PSSA bacteremia. MRSA bacteremia was more often associated with CVC than MSSA bacteremia (OR 2.10, 95% CI 1.27-3.47). MRSA bacteremia was more often associated with SSTI than MSSA (OR 1.52, 95% CI 0.99-2.33) or PRSA bacteremia (OR 1.70 95% CI 1.07-2.69) but no statistically significant difference was noted when compared to PSSA bacteremia (OR 1.23 CI 95% 0.73-2.06). The focus remained equally unknown in all subgroups. There was no significant difference in foci between PRSA and PSSA bacteremias.

Table 6. Focus in MRSA, MSSA, PRSA and PSSA bacteremia. Percentages are computed as the per cent of all non-missing values.

	MRSA	MSSA	PRSA	PSSA
Focus	n (%)	n (%)	n (%)	n (%)
Pneumonia	24 (20)	57 (16)	34 (14)	23 (19)
Skin or soft tissue infection	56 (46)	131 (36)	83 (35)	48 (40)
Deep abscess	8 (7)	29 (8)	17 (7)	12 (9)
Native arthritis	4 (3)	23 (6)	17 (7)	6 (5)
Central venous catheter	35 (29)	59 (17)	37 (16)	22 (18)
Infected foreign body[a]	15 (12)	36 (10)	23 (10)	13 (11)
Endocarditis	5 (4)	25 (7)	18 (7)	7 (6)
Spodylodiscitis or osteomyelitis	11 (9)	48 (13)	31 (12)	17 (13)
Mediastinitis	1 (1)	17 (5)	10 (4)	7 (6)
Unknown focus	19 (16)	65 (18)	46 (19)	19 (16)

[a]Infected foreign bodies: 10 vascular prosthesis, 9 osteosynthesis material, 7 pace makers, 25 articular prosthesis

PSSA=Penicillin-susceptible *Staphylococcus aureus, PRSA*=Penicillin-resistant *Staphylococcus aureus*, MRSA=Methicillin-resistant *Staphylococcus aureus*

5.12 Factors associated with mortality in *Staphylococcus aureus* bacteremia (II)

Association of the clinical characteristics of SAB with D28 mortality in the case-control study was analysed for variables presented in the materials and methods section. Variables that were either statistically significant in univariate analysis or considered clinically important were entered into the multivariable model. Of recurrent SAB episodes, only the first was included in the mortality analysis.

In the univariate analysis, methicillin resistance (OR 2.00, 95% CI 1.20-3.34), male gender (OR 1.05, 95% CI 1.02-1.07), cardiac disease (OR 3.27, 95% CI 1.90-5.64), chronic kidney disease (OR 1.63, 95% CI 0.99-2.68), liver cirrhosis (OR 2.17,

95% CI 0.98-4.77) and previous glucocorticoid therapy (OR 2.17, 95% CI 1.28-3.67) were significantly associated with D28 mortality. Appropriate antimicrobial therapy was a protective factor for D28 mortality in univariate analysis (OR 0.60, 95% CI 0.34-1.06). These variables and two additional variables considered clinically relevant (chronic obstructive pulmonary disease or asthma and diabetes) were entered in the multivariable analysis.

In the multivariable analysis, methicillin resistance (OR 2.34, 95% 1.02-5.37), age (OR 1.05, 95% CI 1.02-1.08), cardiac disease (OR 1.86, 95% CI 1.00-3.44), liver cirrhosis (OR 3.10, 95% CI 1.22-7.85) and previous glucocorticoid therapy (OR 2.22, 95% CI 1.23-4.00) were significantly associated with D28 mortality. Appropriate antimicrobial therapy (OR 1.33, 95% CI 0.53-3.33) was not a protective factor for D28 mortality in multivariable analysis.

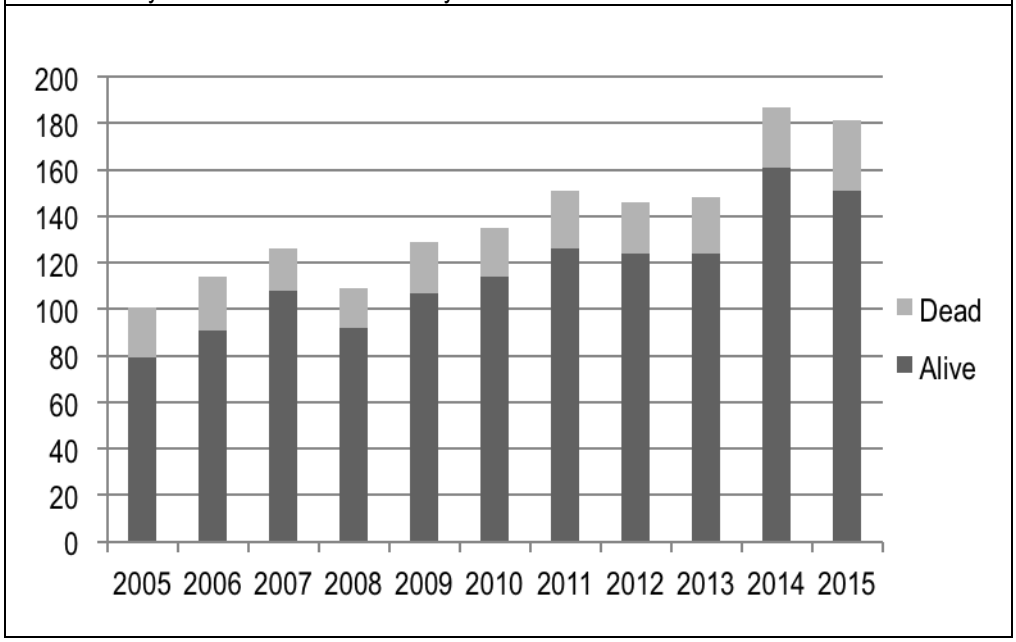
5.13 Focus and mortality in SAB (II)

The association of focus of SAB with D28 mortality was assessed. Pneumonia was associated with higher D28 mortality (OR 2.51, 95% CI 1.42–4.44) but SSTI (OR 0.42, 95% CI 0.24–0.74) and osteomyelitis or spondylodiscitis (OR 0.32, 95% CI 0.11–0.90) with lower mortality. An unknown focus was associated with higher mortality (OR 1.78, 95% CI 0.98–3.23). No other associations between infection foci and D28 mortality were found (deep abscess, native arthritis, CVC-associated bacteremia, other infected foreign body, endocarditis and mediastinitis).

5.14 Outcome of *Staphylococcus aureus* bacteremia (II and IV)

Outcome of SAB was analysed in a population based setting in study IV and in a case control cohort in study II. The number of cases who survived and died in SAB in the population based study is shown in Figure 7. The overall case fatality rate was 21.8% in 2005, 20.2% in 2006 and has since stabilized at a level of 13.9%-17.1%.

Figure 7. Number of *Staphylococcus aureus* bacteremia cases who died and survived within 28 days from the blood culture in years 2005 to 2015.



In the population-based study (original article IV, Table 2), D28 mortality was higher among MRSA cases (26.2%, 34/130) than PRSA (16.3%, 151/924) or PSSA 13.7, 65/473) cases. Day 90 (D90) mortality was also highest in MRSA bacteremia (MRSA 40.8%, 53/130; PRSA 22.9%, 212/924; PSSA 20.7, 98/473). D28 and D90 did not differ significantly between PSSA and PRSA cases. D28 and D90 mortality differed significantly between infections with MRSA and MSSA (26.2 % vs 15.5%, $p=0.002$ and 40.8% vs 22.2 %, $p<0.001$, respectively).

In the case control study D28 mortality in MRSA bacteremia was 26.8% (30/112), in MSSA bacteremia 15.5 (52/336), in PRSA bacteremia 17.0 (38/224) and in PSSA bacteremia 12.5% (14/112). Recurrent bacteremia episode were excluded from the mortality analysis.

ORs for differences in D28 mortality between MRSA, MSSA, PRSA and PSSA bacteremia (study II) are presented in Table 7. ORs were also adjusted for underlying conditions and for foci of infection. The underlying conditions that were found to be significant in univariate analysis were entered into the model. D28 mortality in MRSA bacteremia appears to be higher than in MSSA, PRSA or PSSA bacteremia also when adjusted with underlying diseases and foci of infection. There was no difference in D28 mortality between PRSA and PSSA.

Table 7. Odds ratios and 95% confidence intervals for a comparison of D28 mortality in MRSA, MSSA, PRSA and PSSA bacteremias adjusted for underlying conditions and foci.

	MRSA vs. MSSA	MRSA vs. PRSA	MRSA vs. PSSA	PRSA vs. PSSA
D28 mortality	2.00 (1.20-3.34)	1.79 (1.04-3.09)	2.56 (1.27-5.15)	1.43 (0.74-2.77)
D28 mortaltity, adjusted for underlying diseases	2.00 (1.16-3.45)	1.81 (1.00-3.26)	2.60 (1.27-5.33)	1.56 (0.78-3.15)
D28 mortaltity, adjusted for foci of infection	2.32 (1.33-4.01)	1.99 (1.12-3.56)	3.02 (1.43-6.38)	1.66 (0.79-3.48)

PSSA=Penicillin-susceptible *Staphylococcus aureus, PRSA*=Penicillin-resistant *Staphylococcus aureus*, MSSA=metchicillin-susceptible Staphylococcus aureus, MRSA=methicillin-resistant *Staphylococcus aureus,* D28= day 28

5.15 Treatment of *Staphylococcus aureus* bacteremia (II)

Treatment of MRSA, MSSA, PRSA and PSSA was studied (Original article II, table III). Altogether 31% (37/118) of MRSA cases and 98% (349/358) of MSSA controls received adequate empiric antibiotics ($p<0.001$). Of MRSA cases 67% (85/126) were previously known carriers of MRSA and 40% (34/85) of them received appropriate empiric antibiotic. Data on the empiric antibiotic treatment were missing from 6% (28/504) of cases.

Echocardiogram (either transthoracic or transoesophageal) was performed for 28% of MRSA cases and for 47% of MSSA cases. The difference was statistically significant ($p<0.001$).

Of MRSA and MSSA cases, 12 % and 14% were treated in the ICU, respectively. Thirteen percent of MRSA cases and 8% of MSSA cases needed invasive ventilation. The difference in these variables was not statistically significant ($p=0.663$ and $p=0.279$, respectively).

MRSA cases needed acute renal replacement therapy more often than MSSA cases (6% vs 1%, respectively, $p=0.001$).

5.16 Spa type distribution in MRSA and MSSA bacteremia (III)

In study III, spa type distribution in MRSA (n=109) and MSSA (n=353) bacteremias were analysed in years 2004-2013. The spa type distribution in MRSA bacteremias and the most common spa types in MSSA bacteremias (n≥4) are shown in Table 8.

Among MRSA bacteremias, 14 different spa types were found. The most common spa type was t067 (n=90/109, 82.6%). There were four cases of t032 (4/109, 3.7%), three cases of t172 (3/109, 2.8%) and two cases of t3109 (n=2/109, 1.8%). Only single cases caused by other spa types, namely t008, t015, t022, t6450, t9628, t976, t1955, t3148, t4906 and t11462, were found among MRSA bacteremias.

In MSSA bacteremias, 154 different spa types were found. The most common spa types (n≥10) were t172 (n=28/353, 7.9 %), t084 (21/353, 5.9%), t015 (20/353, 5.7%), t026 (n=17/353, 4.8%), t160 (n=14/353, 4.0 %), t267 (n=11/353, 3.1%), t008 (n=11/353, 3.1%) and t002 (n=11/356, 3.1%), respectively. There were 113 singletons.

Four spa types (t067, t172, t015 and t008) were found both in MRSA and in MSSA bacteremias while all the other spa types were found only either in MRSA or in MSSA bacteremias.

Table 8. Ranking of the spa types found in MRSA bacteremias and the most common spa types found in MSSA bacteremias

MRSA			MSSA		
Spa type	n	%	Spa type	n	%
t067	90	82.6	t172	28	7,9
t032	4	3.7	t084	21	5,9
t172	3	2.8	t015	20	5,7
t3109	2	1.8	t026	17	4,8
t015	1	0.9	t160	14	4
t008	1	0.9	t267	11	3,1
t022	1	0.9	t008	11	3,1
t11462	1	0.9	t002	11	3,1
t1955	1	0.9	t021	9	2,5
t9628	1	0.9	t127	9	2,5
t976	1	0.9	t164	7	2
t3148	1	0.9	t302	6	1,7
t6450	1	0.9	t012	6	1,7
t4906	1	0.9	t065	4	1,1
-	-	-	t085	4	1,1
-	-	-	t1012	4	1,1
-	-	-	t346	4	1,1
-	-	-	t519	4	1,1

MRSA=Methicillin-resistant *Staphylococcus aureus,* MSSA= Methicillin-susceptible *Staphylococcus aureus*

Spa type distribution in PRSA and PSSA invasive strains is presented in Table 9. The most common spa type in both groups accounted for almost one tenth of the cases, but no such dominating strain as in MRSA is found amongst PRSA or PSSA. Spa types t0172, t015, t026 were common in both PRSA and PSSA.

Table 9. Most common spa types in PRSA and PSSA bacteremias

PRSA			PSSA		
Spa	n	%	Spa	n	%
t172	23	9,7	t160	11	9,4
t084	19	8,1	t002	9	7,7
t015	13	5,5	t015	7	6
t026	11	4,7	t026	6	5,1
t021	9	3,8	t267	6	5,1
t008	7	3	t172	5	4,3
t164	7	3	t008	4	3,4
t012	6	2,5	t065	4	3,4
t127	5	2,1	t127	4	3,4
t267	5	2,1	t519	4	3,4
t085	4	1,7	t067	3	2,6

PSSA=Penicillin-susceptible *Staphylococcus aureus, PRSA*=Penicillin-resistant *Staphylococcus aureus*

Since the adoption of the spa typing method as a primary typing method in 2009 to the end of year 2013, 1645 positive MRSA screening samples from non-infected carriers were detected (Original article III, Table 2). Among them, 115 different spa types were found. For 14 carriers, the spa type was not available. The most common spa types (n≥10) in the screening samples were t067 (61.8%, 1017/1645), t172 (6.4%, 105/1645), t008 (6.3%, 104/1645), t032 (3.0%, 50/1645), t1012 (3%, 50/1645), t596 (1.3%, 21/1645), t026 (1.2%, 20/1645), t3109 (1.1, 18/1645), t002 (1.0%, 17/1645), t022 (0.8, 13/1645).

During years 2009 to 2013, 58 MRSA bacteremias were diagnosed and spa typed. Of the most common spa types in the screening samples, five were also found in bacteremic strains: t067 (79.3%, 46/58), t032 (6.9%, 4/58), t172 (3.4%, 2/58), t008 (1.7%, 1/58) and t3109 (1.7%, 1/58). The common spa types t1012, t596, t026, t002 and t022 in screening samples were not found in bacteremic strains during that period.

Although spa typing is usually performed each time *S. aureus* is recovered from blood culture, we found 19 cases during 2009-2013 in which the typing had not been performed at the time of bacteremia as they were already known MRSA carriers. Those strains were spa typed within this study and three cases were regocnized in which the spa type of the colonizing strain was different than that of the invasive strain recovered years later. Two t067 carriers had bacteremia caused by t032 strain, and one t067 carrier had bacteremia caused by t11462 strain.

5.17 Assignment of spa types in spa clonal complexes (III)

The assignment of each spa type in spa clonal complexes (spaCCs) and their occurrence in bacteremic *S. aureus* strains (MRSA and MSSA combined) is presented in Table 10. None of the spaCCs include only MRSA strains but there were five spaCCs including only MSSA strains, namely spaCC 084, spaCC 164, spaCC 012, spaCC 2133 and spaCC 081. SpaCC could not be defined for 20 spa types with fewer than five repeats, four spa types were considered non-founders and 10 were singletons.

Table 10. The assignment of each spa type in spa clonal complexes (spaCCs) and their occurrence in bacteremic *Staphylococcus aureus* strains

Spa clonal complex	Spa types	n (%) of cases
spaCC 067	t067, t002, t105, t010, t088, t11462, t14809, t2532, t3109, t3134, t3148, t509, t548, t688, t9110	121 (26.2)
spaCC 630	t015, t302, t073, t1510, t050, t102, t230, t4488, t550, t630, t1122, t116, t14628, t14811, t14815, t14818, t2120, t2171, t2626, t295, t487, t583, t6406, t908, t065, t1012, t1402, t040, t11344, t1248, t130, t14814, t14821, t2143, t2275, t507, t6450, t715, t9628	79 (17.1)
spaCC 084	t084, t085, t346, t091, t491, t5387, t094, t14819, t14865, t2623, t3024, t358, t547, t803	44 (9.5)
spaCC 164	t267, t127, t164, t731, t1236. t1429, t14630, t1497, t1508, t164, t16555, t174, t189, t2094, t3004, t4158, t521, t8225	42 (9.1)
spaCC 172	t172, t9629, t976	33 (7.1)
spaCC 012	t021, t012, t018, t090, t14810, t14816, t238, t275, t298, t342, t382, t710	25 (5.4)
spaCC 008	t008, t024, t064, t14817, t1617, t211, t304, t574, t596, t6837, t711, t7840	23 (5.0)
spaCC 2133	t160, t14629, t14820, t156, t2133, t888	19 (4.1)
spaCC 081	t078, t1102, t056, t081, t087, t2078, t660	10 (2.2)
spaCC 005	t022, t032, t005, t1955, t1891	8 (1.7)
Singletons	t100, t136, t148, t488, t647, t1458, t2095, t10471, t14631 t14813	11 (2.4)
Nonfounders	t159, t377, t645, t14812	6 (1.3)
Excluded	t026, t519, t287, t693, t1023, t132, t14721, t1509, t282, t362, t3812, t3929, t4571,t4906, t52, t559, t5909, t650, t779, t8543	41 (8.9)

5.18 Association of spa type and spa clonal complex with the clinical characteristics of bacteremia (III)

Association of spaCC and clinical characteristics of bacteremia is presented in Table 11. In this analysis MRSA and MSSA cases are combined. SpaCC008 was more often associated with endocarditis than other spaCCs (5/23 (21.7%) vs. 21/419 (5,0%), p=0.008), and spaCC 012 with foreign body infections than other clonal complexes (6/24 (25.0%) vs. 43/420 (10.2%), p=0.038), respectively. SpaCC 2133 was more often associated with SSTI than other clonal complexes (13/19 (68.4%) vs. 162/422 (38.4%), p=0.014). CVC associated infection was more common in bacteremias caused by spaCC067 than other strains (30/117 (25.6%) vs 5/324 (15.7%), p=0.025). All spa CCs were found both in CA and in HA bacteremias.

Table 11. Association of spa clonal complex (spaCC) with clinical characteristics of *Staphylococcus aureus* bacteremia

	spaCC 005 n (%)	spaCC 164 n (%)	spaCC 084 n (%)	spaCC 012 n (%)	spaCC 067 n (%)	spaCC 008 n (%)	spaCC 081 n (%)	spaCC 2133 n (%)
CVC associated infection	1 (13)	7 (18)	5 (12)	2 (8)	30 (26)	3 (13)	1 (11)	4 (21)
Deep abscess	0 (0)	3 (7)	5 (12)	2 (8)	11 (9)	1 (4)	0 (0)	2 (11)
Endocarditis	0 (0)	4 (10)	2 (5)	0 (0)	5 (4)	5 (22)	0 (0)	1 (5)
Infected foreign body	0 (0)	2 (5)	3 (7)	6 (25)	17 (15)	1 (4)	2 (22)	3 (16)
Pneumonia	1 (13)	9 (22)	6 (15)	6 (25)	19 (16)	3 (13)	1 (13)	3 (16)
Spondylodiscitis or osteomyelitis	1 (13)	7 (17)	6 (14)	3 (12)	11 (9)	2(9)	1(10)	1 (5)
SSTI	2 (25)	14 (34)	12 (29)	10(42)	57 (49)	6 (26)	3 (38)	13 (68)
Tromboembolic event	0 (0)	5 (12)	5 (11)	4 (16)	11 (9)	1 (4)	0 (0)	1 (5)
Current smoking	1 (14)	5 (14)	7 (20)	4 (17)	27 (34)	2 (13)	2 (22)	4 (27)
HA	6 (75)	26 (63)	18 (41)	15 (65)	91 (76)	12 (52)	4 (50)	12 (63)

CVC=central venous catheter, SSTI=skin and soft tissue infection, HA= health care associated

5.19 Spa type, spa clonal complex and mortality (III)

Day 28 mortality in relation to spaCC and spa type is shown in Table 12. D28 mortality was the highest in spaCC 067 (31/121, 25.6%). This difference in mortality was statistically significant when compared to other spaCCs combined (52/34 (15.2%), p=0.011). D28 mortality across the most common (n>10) spa types is also shown. D28 mortality was highest in t002 and t067 bacteremias.

Table 12. Day 28 mortality in relation to spa clonal complex (spaCC) and spa type. The most common spa types (n≥10 are shown)

	n	total	%	spa type	n	total	%
spaCC 067	31	121	25.6	t002	3	11	27.3
spaCC 008	5	23	21.7	t067	25	93	26.9
spaCC 630	16	79	20.3	t015	5	21	23.8
spaCC 164	8	42	19	t084	3	21	14.3
spaCC 084	8	44	18.2	t026	2	17	11.8
spaCC 012	4	25	16	t172	3	31	9.7
spaCC 005	1	8	12.5	t267	1	11	9.1
spaCC 172	3	33	9.1	t008	1	12	8.3
spaCC 2133	1	19	5.3	t160	1	14	7.1
spaCC 081	0	10	0	-	-	-	-

6 DISCUSSION

6.1 MRSA transmissions

The study I showed a significant reduction in HA-MRSA transmissions in Pirkanmaa county since 2011. There may be several explanations for this. Infectious disease epidemics tend to rise and fall for reasons that are poorly understood. In the case of MRSA, a similar phenomenon in MRSA dynamics was seen in the UK already before comprehensive infection control measures were established [178,179]. The authors suggested that some indeterminate property of the organism, or an acquisition of immunity in the population contribute to the end of the transmission wave [179].

In Pirkanmaa, timely association with improvements in the level of hospital hygiene and decline of HA-MRSA transmissions was noted. The decrease in MRSA transmissions did not start until after the year 2011. Several hospital hygiene interventions were established stepwise over the years in the health care units of Pirkanmaa county. Adherence was ensured by continuous surveillance and feedback given to the health care units by IU's ID-specialists, IC-nurses and link nurses and the MRSA Working Group. Until the year 2008 the number of MRSA transmissions increased although targeted screening was already launched in 2001, link nurse network was established in 2004 in LTF and primary care and an interim infection control campaign was organized in 2004 in LTF and 2009 in TAUH. Probably these interventions were not sufficient. Interim campaigns do not improve the level of hospital hygiene for the long term. From the beginning of the 2010s, more resources were steered for hospital hygiene work, which made it possible to hire more infection control staff. Moreover, universal screening was launched in 2011. As several studies have shown the efficacy of infection control measures on MRSA transmission rates, it is likely that combined and sustained hospital hygiene interventions largely explain the decrease of HA-MRSA transmissions in Pirkanmaa [128,129,151,155,180].

Increased use of alcohol hand-rub serves as a surrogate marker of improved hand hygiene. The use of hand-rub more than doubled in TAUH. The number of MRSA cases started to decrease only after the hand-rub consumption exceeded

92.5 L/1000 patient days. Hand hygiene has been promoted by repetitive educational visits by IC-nurses and ID-specialists as well as hand hygiene campaigns in TAUH, SHs, LTF and health centres. Since 2012, hand hygiene compliance has been continuously monitored by link nurses in TAUH wards. This probably also had a beneficial effect as performance feedback is known to improve hand hygiene compliance [181]. Hand hygiene is thought to be the single most effective MRSA control measure and necessary for the success of other policies [129,160] although some studies have reported that a 60% increase in hand hygiene compliance reduces the MRSA prevalence by only 20% [182]. Based on our results, we may hypothesize that hand-rub consumption must exceed a certain critical level before its effect on MRSA rates is seen.

As the screening recommendations expanded, the number of annual MRSA screening samples increased remarkably from 48 000 in screening period I to 228 000 in screening period III. It has been suggested that targeted screening misses 45% of MRSA carriers at admission. On the other hand, with targeted screening policy, 75% fewer patients would have been screened which reduces costs of screening [183]. Fuller et al have reported that checklist activated screening would detect 80% of the MRSA positive patients detected by universal screening of all admissions, whilst halving the number of laboratory screens [184]. The advantages and cost effectiveness of screening strategy obviously require consideration of local MRSA prevalence and epidemiology. Employees' commitment to targeted screening may also be challenging, as they have to go through a complicated checklist of MRSA risk factor with every admitted patient. Moreover, some MRSA carriers do not have recognized risk factors, especially those with CA-MRSA [184]. Thus, universal screening may be associated with better screening compliance.

The epidemic has been mainly caused by a single strain, t067. Chracteristics of the strains may play a role in the wave trajectory the epidemic. Expansion and decline of t067 coincide with the dynamics of the whole epidemic. It has been suggested that an epidemic caused by a single strain may refer to a single source and relative fitness of the strain while MRSA epidemics caused by several strains rather refer to poor level of hospital hygiene [41]. The t067 is also the most common strain in Zaragoza, Spain, which supports the theory of some strains ability to spread more efficiently than other strains [185].

Early recognition of MRSA carriers does not only serve the purpose of infection control. As MRSA colonisation usually precedes infection, awareness of patient´s MRSA carriage may facilitate the choice of correct empiric antibiotic when a patient develops a serious infection. It also provides an opportunity to give

the MRSA-positive patient decolonisation therapy. Correct choice of empiric antibiotic may improve the outcome of MRSA bacteremia although in our study (II) we could not confirm this [117,186,187]. In our study 31% of MRSA patients received appropriate empiric therapy which is a lower percentage that in the reviewed literature [117,118] .

In the first two years of the Pirkanmaa MRSA epidemic, majority of new MRSA findings were from clinical samples. As MRSA infections represent only the tip of an iceberg of the MRSA distribution, it is likely that the epidemic was already in the early years more widely disseminated than what was realised. Large numbers of unrecognized MRSA carriers were at risk of not having appropriate empiric treatment for their MRSA infections. Additionally, unrecognized MRSA carriers were not treated with contact precautions in hospital wards and LTF, which led to further transmissions. Although universal screening is used, a part of new MRSA cases are still found in clinical samples, as the infection can be the first manifestation of MRSA carrier state. In our study 15% of all new MRSA findings were recovered from clinical samples even during screening period III. In comparison, a Swedish study reported that 36% of new MRSA findings were recovered from clinical samples in an area where targeted screening was deployed [188]. Nevertheless, our results showed that even when MRSA carriage status was known, only 40% of MRSA carriers received appropriate empiric antibiotic for MRSA bacteremia.

In conclusion, improved hospital hygiene and universal screening lead to a significant decrease in MRSA transmissions. The commitment of staff and administration to preventing HA infections and sustained and combined interventions is vital in the prevention of MRSA transmissions. Early recognition of MRSA carriers increases the probabiblity of choosing appropriate empiric antibiotic when MRSA infection develops. Natural strain dynamics also contribute to the rise and fall of MRSA epidemics.

6.2 Incidence of *Staphylococcus aureus* bacteremia

The proportion of MRSA in SAB is considered to be a valid indicator of MRSA control because it is not influenced by screening activity [189]. In study IV it was seen that along with the decrease of MRSA transmissions in Pirkanmaa (study I), the indicence of MRSA bacteremias declined significantly after the year 2011. As MRSA transmissions have decreased, also the number of people at risk of MRSA

bacteremia has decreased. Decolonization therapy recommened to known MRSA carriers since 2013 may also have decreased the incidence of MRSA bacteremia, but its impact is unknown since we do not know the number of decolozation therapies given. The incidence of MRSA bacteremia decreased during the study period to the same level as in other parts of the country. This implies that the prevalence of MRSA carriage in Pirkanmaa is probably no longer higher than in other parts of the country. As this study was of population-based design, it provides the least biased assessment of the disease burden.

We hypothesized that improved hospital hygiene would lead to concurrent reduction of HA-MSSA bacteremias, but we found that both CA and HA-MSSA bacteremias increased. This is contrary to findings from Australia, where 9.4% decrease in the incidence of HA-MSSA bacteremias was noted as the level of hospital hygiene improved [190]. Our findings are in line with observations of Lawes et al, who noted in the UK that the effect of improved hospital hygiene and universal MRSA screening on SAB incidence is MRSA specific [137].

The increasing burden of SAB has been also reported by other authors [78,81,191]. There are several explanations to this. The most important reason for the increase of the CA-MSSA cases is probably improved diagnostics. The number of blood screens taken annually increased remarkably, by over 40% during the study period, while the rate of positive *S. aureus* findings per 10 000 blood cultures remained stable. This indicates that during the early years of the study period SAB may have been underdiagnosed. This change in blood screening activity is probably attributed to the reformation of acute care. During the study period 2005-2015, several emergency units in health centres were shut, and acute care was centralized to TAUH and Valkeakoski secondary hospital. This may have improved the diagnostics of severe infections. An increase in the rate of blood culture sampling by 21% was already reported in Finland during the years 1995-2001 [81].

The increase of both CA-MSSA and HA-MSSA bacteremias may also have resulted from the ageing of the population. The overall incidence and the incidence of HA bacteremia is highest in the oldest age groups. The population ≥75 years rose during the study period in Pirkanmaa from 36 251 to 44 326 [192]. Due to the progress of medical treatment, the number of invasive procedures and use of intravascular devices have probably increased and accounted for the increase of HA bacteremias. Although we did not find any favorable effect of improved hospital hygiene on HA-MSSA incidence, we may postulate, that the HA-MSSA incidence might be ever higher if the level of hospital hygiene was lower considering the probable increase in the number of people at risk of bacteremia.

It is also possible that a new successful MSSA strain is spreading and causing an epidemic as suggested by Resman et al [30]. However, our finding in study III does not support this theory, as there does not appear to be a single dominant strain among invasive MSSA strains.

The first *S.aureus* strains producing penicillinase appeared soon after penicillins were introduced and they rapidly became the prevalent *S. aureus* strain [20]. In our study, the proportion of PSSA in invasive findings increased constantly during the study period reaching the proportion of 43.1 % of all SAB in the end of the study period, although PRSA still accounted for the majority of the cases. There are reports of similar trends from the USA [32,124] but opposite in Sweden [90]. The reason for the increase of PSSA strains is unknown. Crane et al discussed that the reason for the increase in PSSA strains noted in New York may be a heavy reliance on vancomycin, which may have created a niche for these strains [32]. This is not a probable explanation for our findings, as vancomycin is not used in the empirical treatment of sepsis in Pirkanmaa. It has not been recommended in empiric therapy in Pirkanmaa even in the most challenging years of the epidemic except in patients who are known MRSA carriers in whom it is combined with a cephalosporin. PRSA was more common in CA than in HA cases. This may be related to a more abundant use of penicillin and amoxicillin in primary care as compared to hospitals. Cephalosporins are commonly used in hospitals, which may also contribute to the relative success of PSSA strains as cephalosporins have been reported to be inferior to penicillin and cloxacillin in the treatment of PSSA infections [125]. In this propensity-score-matched case-control study, the 30 day mortality was 39% in patients treated with cefuroxime treatmen versus 20% in patients treated with penicillin ($p=0.037$). In the comparison of cefuroxime versus dicloxacillin treatment the difference was also significant (38% vs. 10%, respectively, $p=0.004$).

As a consequence of the high prevalence of PRSA strains, many laboratories have discontinued the testing of penicillin susceptibility years ago. Based on our results we encourage laboratories to restart the testing. Within the era of growing antibiotic resistance, it must be kept in mind that penicillin in the treatment of PSSA could theoretically reduce the selection pressure that favours the spread of MRSA strains [20]. Penicillin has advantages over cloxacillin and cephalosporins in the treatment of PSSA bacteremia [125]. It is relatively cheap, causes less irritation of the cannulated vein and the MIC values are lower for penicillin than for cloxacillin [82]. Plasma concentrations of penicillin are higher than those of cloxacillin with conventional doses [90]. Suboptimal plasma concentrations of betalactams are a risk especially in obese patients, critically ill patients with

augmented renal clearance or young children [193]. Penicillin is less associated with the *Clostridium difficile* infection than broader-spectrum antibiotics [194]. Chabot et al reported that penicillin was used in only 0% - 50% of PSSA bacteremia cases during 2003-2012 in Massachusetts [124]. Penicillin is probably underused in the treatment of PSSA bacteremia also in Finland. Betalactams may also have some disadvantages, as suboptimal plasma concentration are known to up-regulate several virulence factors, while linezolid and clindamycin down-regulate most of them [193]. This phenomenon may contribute to an undesirable outcome in toxin mediated *S. aureus* diseases.

In conclusion, SAB appears to cause a continuously growing burden on the population. However, during recent years, the most common resistance patterns of *S. aureus*, namely penicillin and methicillin resistance, are declining in invasive isolates in Pirkanmaa county. This contradicts the common assumption that resistant strains are gradually replacing susceptible strains. Testing of penicillin susceptibility should be re-stared by laboratories that have discontinued it, as it enables the use of narrow spectrum antibiotics.

6.3 Risk factors of MRSA bacteremia

In study II we found that MRSA bacteremia patients were more often smokers and on glucocorticoid therapy than MSSA bacteremia patients. MRSA was also more often associated with hospital acquisition and previous surgery, which was in line with previous studies [94,195].

Smoking is known to increase the risk of *S. aureus* nasal carriage [196] and second-hand smoke exposure in infants with cystic fibrosis is associated with MRSA colonization [197]. In Chinese children, passive smoking has been shown to be associated with MRSA nasal colonization [198]. Cigarette smoke increases the adherence of MRSA to epithelial cells as well as its invasion of epithelial cells and MRSA colonies exposed to cigarette smoke extract is shown to be more resistant to macrophage killing [199]. Smoking also increases *S. aureus* biofilm formation [200]. Some studies have reported that smoking might be associated with non-invasive MRSA infections [201,202], but there are no earlier reports of an association between smoking and MRSA bacteremia.

In study II, glucocorticoid therapy was more common in patients with MRSA bacteremia than in patients with MSSA bacteremia. It has been reported earlier,

that glucocorticoid therapy was associated with HA-SAB but the association with MRSA bacteremia was not reported in that study [203]. Another study reported that patients with CA-MRSA infection or colonisation were more often on glucocorticoid therapy than age-matched controls [204]. In patients with systemic lupus erythematosus glucocorticoid therapy is a risk factor for infections caused by multidrug-resistant bacteria, including MRSA [205]. Most studies do not assess the association of a glucocorticoid therapy with MRSA infections, as only association of heterogeneously defined immunosuppressive status and infections caused by multidrug-resistant bacteria have been reported.

In conclusion, previous surgery, HA, smoking and glucocorticoid therapy were associated with MRSA bacteremia as compared to MSSA. There was no significant difference in the risk factors between PRSA and PSSA bacteremia.

6.4 Mortality in SAB

D28 mortality in MRSA bacteremia was higher than in MSSA, PRSA and PSSA bacteremia both in the case-control study (study II) and in the population based study (study IV). The difference remained even when mortality was adjusted with underlying diseases and focus of bacteremia. This is in line with previous studies [87]. However, the resistance profile *per se* may not be the only factor influencing the prognosis. There are probably a number of other factors determining the outcome as well. Although in the case-control study (II) MRSA and MSSA cases were gender- and age-matched, it is likely that MRSA cases had more severe underlying diseases, as HA bacteremia was more common in MRSA cases.

Appropriate empiric treatment was a protective factor from death within 28 days but was not significant in multivariable analysis adjusted for underlying conditions. In several studies, inappropriate empiric therapy was the most important predictor of death in MRSA bacteremia [117,186,187]. On the contrary, Kaash et al and Jacobsson et al showed that in patients with SAB early appropriate antimicrobial therapy may not improve survival [91,206]. The probable explanation for our findings is that survival is strongly influenced by patient characteristics and duration of symptoms before diagnosis. We did not analyse the delay in the treatment. It is also known that certain antibiotics, although effective in vitro and in high plasma concentrations, may increase endotoxin production of *S. aureus* at sub-inhibitory concentrations. This may play a significant role in the disease course

of toxin-mediated diseases such as toxic shock syndrome and necrotizing pneumonia. This is especially shown with cell wall-active betalactams [193].

Of the focus of infection, SSTI and osteomyelitis or spondylodiscitis were associated with lower D28 mortality. This may be due to the specific symptoms from these foci, which may lead to earlier diagnosis and treatment. Pneumonia and unknown focus were associated with higher D28 mortality. Our findings are in line with previous studies [91,92,97,186]. Focus may remain unknown due to insufficient investigations, which may lead to insufficient source control and have implications on the outcome. It is for example possible in our cohort that some endocarditis cases were missed because echocardiography was performed only in 28% of MRSA cases and 47% of MSSA cases. In comparison, echocardiogram was performed in 57% of SAB patients in a study by Kaasch et al [92] and in 50.6% of SAB patients in a study by Asgeirsson et al [207].

The difference in mortality between PRSA and PSSA did not reach statistical difference although PRSA mortality appears to be slightly higher. These findings are in line with other studies [82,90]. Bigger sample size is required to address this possible difference.

Association of spa type, spaCC with D28 mortality in SAB was also studied. The highest mortality was in bacteremias caused by t002 or t067 or by strains belonging to spaCC 067. The high incidence of MRSA t067 in long-term facilities probably explains its association with mortality. SpaCC 067 also mainly consists of MRSA t067, which explains the relatively high mortality associated with this SpaCC. Interestingly, both t067 and t002 belong to the same spaCC 067. This might indicate that these related strains share virulence factors determining the disease severity.

In the study IV we noted that case fatality in SAB has decreased from 21.8-20.2 to 13.9%-17.1% during recent years. This may indicate that the quality of care of SAB may have improved during the study period in Pirkanmaa. It is also possible, that cases of lesser severity were more frequently detected with higher blood screening rates. Forsblom et al reported an overall D28 case fatality of 13% in Finland in years 1999-2002 in a prospective multi-center study, but their study was a cohort study, not population based as ours, and did not include MRSA patients [97]. In that study, every patient was followed by an ID-specialist, and only in 5% of the cases the focus of infection remained unkwown. Lyytikäinen et al reported D28 overall SAB mortality of 17% in 1995-2001 in a population-based study covering whole Finland. Improved survival of SAB patients in low MRSA prevalence settings has been reported by other authors. In Denmark, case fatality

decreased from 34.5% to 21.7% in years 1981-2000 [77]. In Iceland, all-cause 30-day mortality decreased from 25.0% to 8.1% in years 1995-2008 [78]. Opposite trends were reported by Yahav et al. They reported increasing mortality from 35.7% to 42.9% in years 1988-2010. Although the study was performed in a high MRSA setting, the MRSA rate remained stable over the study period. The increased mortality was explained by more severe underlying conditions of the patients in the latter phase of the study [85].

In conclusion, case fatality has decreased in Pirkanmaa during the study period. Methicillin resistance, unknown focus, pneumonia and spaCC 067 were associated with higher mortality while appropriate antimicrobial therapy and penicillin resistance had neutral effect on mortality.

6.5 Spa type distribution in MRSA and in MSSA bacteremias

We found that spa type distribution in invasive MSSA strains is more diverse than in invasive MRSA strains indicating a closer relatedness of circulating MRSA strains. Amongst MSSA, no such dominating strain was found as t067 in MRSA. As *S. aureus* colonisation usually precedes bacteremia, spa type distribution in blood screens probably reflects the strain diversity of circulating non-invasive strains as well, although some strains appear to have greater tendency to cause invasive infections. The reason for lower diversity of MRSA strains is that MRSA strains are younger in their evolutionary timescales [208]. MRSA also commonly spreads in hospital environment in clonal manner. Consequently, a few successful MRSA strains spreading in hospitals can account to a majority of MRSA findings in a region [209-211]. However, increasing genetic distribution of MRSA has been reported in several studies [212,213].

In Pirkanmaa, the dominant MRSA strain both in screening samples and in invasive samples is t067. It is also known to circulate in Spain and in Southern France indicating that a single, successful strain can cause local epidemics in geographically distant regions [7,8,185]. Spa t067 was even more commonly found in invasive samples than in screening samples. This may suggest, that in addition to the strain´s ability to spread effectively, it may possess some virulence factors that facilitate its invasion. On the contrary, spa t1012 was the fourth most common MRSA finding in screening samples but has not caused any MRSA bacteremias suggesting that this strain may lack some features needed for invasion. The high occurrence of t067 in invasive infections may also be explained by its domination

in Pirkanmaa´s LTF and hospitals where MRSA carriers are more vulnerable to blood stream infections.

Spa t067 was rarely found in MSSA bacteremias, which may reflect its occurrence also in colonizing MSSA strains. If t067 is truly rare among MSSA population, it would be likely that t067 clone acquired its methicillin resistance outside of Finland, was then imported to Finland perhaps by a single source, and has then spread successfully. On the contrary, spa types t172 and t008, which were common spa types in MRSA screening samples, were also common in MSSA bacteremias. These strains may have spread in Finland as MSSA but have later acquired the *mec* element that carries the central genetic determinant for methicillin resistance. It is also possible that they have spread in Finland as MRSA but some lineages have later lost their mec elements or that both the t172 and t008 lineages harboring MRSA and MSSA strains have been imported separately. There is evidence that MRSA strains can regain their methicillin susceptibility by spontaneous excision of the *mec* element [214]. In addition to MRSA, t172 was a common spa type both in PRSA and PSSA bacteremias. This may indicate repeated changes in antimicrobial susceptibility of this strain. Among MSSA, t067 was found exclusively in PSSA cases. In addition, the Pirkanmaa epidemic MRSA t067 strain does not produce penicillinase, (Risto Vuento, personal communication) which further confirms the relatedness of these PSSA and MRSA strains. As our knowledge of spa type distribution of MSSA in Pirkanmaa is only based on bacteremic strains, these results should be interpreted with caution.

Interestingly, there were three cases of MRSA bacteremia, who were previously known MRSA carriers but in whom the bacteremia was caused by different spa type than the colonizing strain. In one of the cases, the strains (t11462 and t067) belonged to the same spaCC indicating their relatedness and intrahost evolution of MRSA [215]. High level of consistency between spa type and phylogenetic position defined by whole-genome sequencing has been demonstrated earlier [216]. In two of the cases the colonizing and bacteremic strains (t067 and t032) may not be related as they were assigned to separate spaCCs. This refers to either the existence of site-specific subpopulations or repeated MRSA transmission.

In conclusion, the rise and fall of MRSA transmissions and bacteremias apper to be strain specific and attributed to t067. In addition to the strains potential capacity to spread efficiently, modest level of hospital hygiene especially in LTF created beneficial circumstances for its dissemination. No other strain has so far reached such ascendancy in Pirkanmaa county. At the present level of hospital hygiene, it is unlikely to happen in the future.

6.6 Spa Clonal Complexes and their association with the clinical characteristics of bacteremia

Providing evidence of the impact of microbial genetic determinants on the clinical course of SAB is challenging due to the complex and dynamic microbe-host interaction. We found several associations between the characteristics of bacteremia and spaCCs. Many of the earlier studies analysing the association of genetic lineage and clinical characteristic have used MLST method for strain distinction. Previous studies have show an association between MLST CC8 and higher mortality [186], CC5 and CC30 and hematogenic complications (native valve endocarditis or hematogenous bone and joint infection) [9] and CC30 and endocarditis [217]. To compare our results to the earlier literature, we used Ridom spa server to map specific spa types to known MLST designations. MLST and spa-typing databases are not fully inclusive, but there are several studies where authors have conducted both spa and MLST determination to demonstrate that a specific MLST is populated by different but closely related spa types [69,218,219]. In our patient population, spa types that correspond to MLST CC30 were grouped into spaCC 012 and were mostly associated with foreign body infections. Spa types corresponding to MLST CC8 were grouped into spaCC 008, which in our study was associated with endocarditis. The only spa type in our study that corresponds to MLST CC5 was t002, which was grouped into spaCC 067 and associated with the highest mortality. Although there is a discrepancy between our results and previous reports, we may postulate that there is a signal that these spaCCs (008, 067, 012) may be associated with complicated and serious forms of SAB. However, each spaCC covers a large diversity of lineages carrying a variety of virulence factors, which makes it difficult to interpret these results.

Although Staphylococcal protein A itself impairs host defence by restraining opsonisation-dependent activation of complement, our results cannot prove that variation in spa gene directly affects the disease course of SAB. It is instead likely, that several virulence factors together impact the outcome and may be linked to certain strains, as other studies have demonstrated [17,106,220]. Interestingly, antimicrobials are known to modulate spa expression [193] and some authors have suggested that this effect might be strain specific [221]. Thus, antimicrobial effect may theoretically be related to spa type.

In conclusion, spa type may modulte the disease course, but the associations we found were weak and may not have significant clinical relevance. There are several

other factors, such as patient characteristics, other virulence properties, resistance pattern and treatment that together determine the outcome of SAB.

6.7 Future considerations

As the proportional value of different hospital hygiene strategies still remain debatable, further assessment is needed to determine the optimal balance between resources used in MRSA control and the benefit gained from these investments in terms of reduction of MRSA rates. This is especially true with the screening policy. The cut-off MRSA prevalence above which the universal screening is cost-effective should be determined [183]. As the majority of disease burden associated with SAB is from MSSA strains, prevention of MRSA infections has only a limited impact on the overall disease burden. The increasing incidence of SAB is partially associated with advancements of modern medicine that require abundant use of catheters and foreign bodies. Even better hospital hygiene measures are needed to restrain the increasing incidense of HA-MSSA bacteremia, for instance by larger scale observation and feedback of hand hygiene and paying more attention to the care of CVCs and wounds. New approaches are also needed to contain the increasing incidence of CA-SAB. Unfortunately, up to this point, *S. aureus* vaccine candidates have failed [222]. Although the outcome of SAB has improved during our study period, even lower mortality would be achieved if all SAB patients received appropriate antibiotic, and echocardiography and consultation of infectious diseases was available for every SAB patient [2].

A trend suggesting that mortality in PRSA bacteremia is higher than in PSSA bacteremia was noted in this study. To address this issue, a study with a bigger sample size is required. Also, a prospective study comparing the outcome and adverse effects in PSSA bacteremia treated with penicillin and staphylococcal penicillin would be interesting. The more common occurrence of PRSA bacteremia in CA might be associated with the widespread use of penicillin in ambulatory care. The comparison of history of antibiotic use of CA-PRSA and CA-PSSA patients would bring light to this matter.

The results of this study are in line with earlier studies suggesting that some *S. aureus* strains possess characteristics that facilitate their successful spread and invasiveness. As some virulence determinants are designated to specific strains, it is probable that *S. aureus* strains also differ in the severity and other characteristics of

the disease they cause. Methods offering higher discrimination power, such as whole genome sequencing, may clarify this issue [216].

7 SUMMARY AND CONCLUSIONS

The main findings in this study were

1. MRSA transmissions in hospitals can be controlled by combined and sustained hospital hygiene interventions. Adherence to these requires commitment from both staff and administration.
2. Incidence of MRSA bacteremia declined as a consequence of decreased MRSA transmissions. MRSA bacteremia is among the most serious infections and it is related with significant mortality. Reduction of the incidence of MRSA bacteremia is the ultimate goal of MRSA control.
3. The burden of MSSA bacteremia for the population continues to grow regardless of improvements in the level of hospital hygiene. It is mainly attributed to ageing and increased co-morbidities of the population and improved diagnostics.
4. Proportion of PSSA in invasive findings is increasing. Testing of penicillin susceptibility enables the use narrow spectrum antibiotics. Use of penicillin in the treatment of PSSA bacteremia reduces the selection pressure that favours the spread of MRSA.
5. Although the incidence of SAB has increased, the mortality per population has remained stable, as the case-fatality ratio has decreased. Thus, both diagnostics and treatment of SAB have improved.
6. Spa type may have an impact on the strains' ability to spread in hospitals, cause invasive disease and modulate the disease course. However, spa may also be only a surrogate marker of strains other virulence properties.

   There are several other factors, such as prevailing level of hospital hygiene, patient characteristics, other virulence factors and treatment that determine the dissemination of *S. aureus* and the disease course of SAB.

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10 APPENDIX

Data collection form

Taustatiedot

1) Koodi [koodi]

2) Nimikirjaimet [nimi]

3) Syntymäaika (muodossa 13.07.2010) [syntaika]

4) Kotipaikkakunta [kotipaik]

5) Sukupuoli [sex]

0) mies

1) nainen

6) Positiivisen veriviljelyn ottopäivä (1.posit.)(muodossa 13.07.2010) [pv0]

7) Staphylococcus aureus [saureus]

0) Herkkä (MSSA)

1) MRSA

8) Tulee lähetteellä [lahete]

0) ei

1) kyllä

2) on jo sairaalassa, ei lähetettä

9) Mistä tulee (sijoituskohde ennen lähetteen tekoa) [mista]

0) kotoa

1) palvelutalosta/vanhainkodista

2) tk-vuodeosastolta

3) aluesairaalasta

4) Tampereen kaupungin sairaalasta (Kauppi tai HASA)

5) On jo TAYSn osastolla

10) Onko tunnettu MRSA-kantaja [kantaja]

0) ei

1) kyllä

11) Sairaalahoidossa tai muussa laitoshoidossa edeltävän vuoden aikana [sairhoi]

0) ei

1) kyllä (TAYS)

2) kyllä (muu sairaala)

3) kyllä (pitkäaikaishoidonlaitos tai vanhainkoti)

4) ei tietoa

12) Jos sairaalahoidossa edeltävän vuoden aikana, montako kk aiemmin viimeisin jakso [sairkk]

13) Alkoholiin liittyen sos/medis.ongelmia [alko]

0) ei

1) kyllä

14) Tupakointi [tupakka]

0) ei koskaan

1) tupakoi

2) tupakoinut, lopettanut

3) tupakointitieto puuttuu

15) Suonensisäisten huumeiden käyttäjä [huumeet]

0) ei

1) kyllä

16) Paino (kg) _____ [paino]

17) Pituus (cm) _____ [pituus]

Perussairaudet

Maligniteetit

18) Solidi maligniteetti [solidima]

0) ei

1) kyllä

19) Metastasoitunut solidi maligniteetti [metasoli]

0) ei

1) kyllä

20) Hematologinen maligniteetti [hemamal]

0) ei

1) kyllä

21) Syöpädiagnoosi, mikä? [syopa]

Diabetes

22) Diabetes, tyyppi I [DM I]

0) ei

1) kyllä

23) Diabetes, tyyppi II [DM II]

0) ei

1) kyllä

Kollagenoosit

24) Reumatoidi artriitti [RA]

0) ei

1) kyllä

25) SLE [SLE]

0) ei

1) kyllä

26) Selkärankareuma [AS]

0) ei

1) kyllä

27) Muu kollagenoosi [muukolla]

Keuhkosairaudet

28) COPD [COPD]

0) ei

1) kyllä

29) Astma [astma]

0) ei

1) kyllä

Munuaistaudit

30) Hemodialyysihoidossa ennen sairastumista [hemod1]

0) ei

1) kyllä

31) Peritoneaalidialyysihoidossa ennen sairastumista [capd]

0) ei

1) kyllä

32) Krooninen munuaissairaus [munuaist]

0) ei

1) kyllä

Maksataudit

33) Maksakirroosi [maksakir]

0) ei

1) kyllä

Sydän- ja verisuonisairaudet

34) Koronaaritauti [koronaar]

0) ei

1) kyllä

35) Sydämen vajaatoiminta [CHF]

0) ei

1) kyllä

36) Perifeerinen ASO-tauti [aso]

0) ei

1) kyllä

Iho

37) Krooninen ekseema [ekseema]

0) ei

1) kyllä

38) Psoriasis [psoriasi]

0) ei

1) kyllä

39) Traumaattinen haava [trauhaav]

0) ei

1) kyllä

40) Krooninen haava [krhaava]

0) ei

1) kyllä

Muut

41) HIV-infektio [HIV]

0) ei

1) kyllä

42) Muu perussairaus [muuperus]

0) ei

1) kyllä

43) Jos muu perussairaus, mikä? [muudg]

44) Vierasesine (0 ei, 1 kyllä) [vierases]

45) Jos vierasesine, mikä? [mikavier]

Infektioalttiusanamneesi

46) Systeeminen kortisonihoito edeltäneen 1 kk aikana

(≥5mg prednisoloni ekvivalenttia/vrk) [kortison]

0) ei

1) kyllä

47) Muu immunosupressiivinen hoito käytössä viimeisen 3 kk aikana (solusalpaaja, siklosporiini tai muu) [immsupp]

0) ei

1) kyllä

48) Jos muu, mikä muu immunosuppressiivinen hoito [immmika]

Postoperatiiviset

49) Operaatio edeltäneen 1 kk aikana [operat]

0) ei

1) kyllä

50) Jos operaatio, mikä? [opermika]

51) Trauma edeltäneen 1 kk aikana [trauma]

0) ei

1) kyllä

52) Jos trauma, mikä? [traumika]

53) Muu bakteremialle altistava toimenpide edeltäneen 1 kk aikana [altistmp]

0) ei

1) kyllä

54) Jos edeltävä tmp, mikä? [altmika]

55) McCabe [McCabe]

0) terve

1) ei-fataali perustauti

2) ultimately fatal (ennuste ½-5v.)

3) rapidly fatal (ennuste <½v.)

Bakteerilöydös

56) Herkkyys kefalotiinille [cef]

0) S

1) I

2) R

57) Herkkyys penisilliinille [pen]

0) S

1) I

2) R

58) Herkkyys erytromysiinille [ery]

0) S

1) I

2) R

59) Herkkyys klindamysiinille [kli]

0) S

1) I

2) R

60) Herkkyys levofloksasiinille [lfl]

0) S

1) I

2) R

61) Herkkyys vankomysiinille [van]

0) S

1) I

2) R

62) Herkkyys sulfatrimetopriimille [sut]

0) S

1) I

2) R

63) Herkkyys tetrasykliinille [tet]

0) S

1) I

2) R

64) Herkkyys tobramysiinille [tob]

0) S

1) I

2) R

65) Herkkyys rifampisiinille [rif]

0) S

1) I

2) R

66) Herkkyys fusidiinihapolle [fus]

0) S

1) I

2) R

67) Herkkyys oksasilliinille [oxa]

0) S

1) I

2) R

Bakteremian taudinkuva

68) Sairaalainfektio (veriviljelylöydös > 48 h sairaalaantulosta) [nosoko]

0) ei

1) kyllä

69) Oireiden kesto ennen veriviljelyä (vrk) [oirekest]

70) Ensioireena kuume [eokuume]

0) ei

1) kyllä

71) Ensioireena kipu [eokipu]

0) ei

1) kyllä

72) Ensioireena yleistilan lasku [eoytlask]

0) ei

1) kyllä

73) Ensioireena sekavuus [eosekava]

0) ei

1) kyllä

74) Ensioireena tajuttomuus [eotaj]

0) ei

1) kyllä

75) Ensioireena hengenahdistus [eohengah]

0) ei

1) kyllä

76) Ensioireena yskä [eoyska]

0) ei

1) kyllä

Statuslöydökset

77) Kuume veriviljelypäivänä (max ◦C) [t0]

78) Verenpaine veriviljelypäivänä (alin mitattu [RRpv0]

79) Tulovaiheen tajunnantaso

0) normaali

1) maininta sekavuudesta tai tajunnan tason laskusta

Bakteremiakohde

80) Bakteremian fokus [fokus]

0) ei tunnettu

1) tunnettu

81) Meningiitti [mening]

0) ei

1) kyllä

82) Pneumonia [pneum]

0) ei

1) kyllä

83) Sappitieperäinen fokus [sappi]

0) ei

1) kyllä

84) Intra-abdominaalinen absessi [abdabses]

0) ei

1) kyllä

85) Osteomyeliitti/spondyliitti/diskiitti [om]

0) ei

1) kyllä

86) Artriitti [artritis]

0) ei

1) kyllä

87) Tekonivelinfektio/vierasesineinfektio [tekonive]

0) ei

1) kyllä

88) Märkivä ihoinfektio (märkivät ihottumat, haavat, fistelit ym) [markiva]

0) ei

1) kyllä

89) Erysipelas tai selluliitti [erysipel]

0) ei

1) kyllä

90) Nekrotisoiva faskiitti/myosiitti/gangreena [faskiitti]

0) ei

1) kyllä

91) Endokardiitti [endokard]

0) ei

1) kyllä

92) Echo tehty [echot]

0) ei

1) TTE

2) TEE

93) Muu infektiolähde/fokus, mikä? [muumika]

Tehohoidon erityispiirteitä

94) Joutuu tehohoitoon (1 kk viljelystä) [tehohoi]

0) ei

1) kyllä, infektion takia

2) teholla jo ennen infektiota

95) Tehohoidon alkupäivämäärä [tehoalku]

96) Tehohoidon loppupäivämäärä [teholopp]

Muut komplikaatiot

97) Tehty revisio tai faskiotomia [revisio]

0) ei

1) kyllä

98) Tehty absessin dreneeraus [dreneer]

0) ei

1) kyllä

99) Tehty proteesin/ vierasesineen poisto [protpois]

0) ei

1) kyllä

100) Emboliset komplikaatiot [embolia]

0) ei

1) kyllä (0-30vrk)

101) Jos embolisia komplikaatioita, mitä? [embomika]

102) Aivoinfarkti seurannassa [CIseur]

0) ei

1) kyllä (0-30vrk)

103) Sydäninfarkti seurannassa [AMIseur]

0) ei

1) kyllä (0-30vrk)

Hoidon lopputulos

104) Outcome, 30vrk viljelystä [outcome]

0) elää

1) kuollut

105) Exituspäivämäärä (pp.kk.vvvv) [exitpvm]

106) Tiedonkeruupv

Antibioottihoito

107) Potilaalla oli jokin antibiootti veriviljelyn ottoaikaan tai 1kk ennen [allaab]

0) ei

1) kyllä

108) Jos menossa antibiootti, niin mikä? [vanhab]

109) Bakteremian aloitusantibiootti tehokas (herkkyysmääritykseen perustuen) [atehokas]

0) ei

1) kyllä

110) Tehokkaan antibiootin aloituspäivämäärä [atehpvm]

111) Mikä aloitus antibiootti? [aloitusab]

Laboratoriokokeet

Päivä 0 (veriviljelyn ottopäivä)

112) Hemoglobiini [Hb0]

113) Trombosyytit [tromb0]

114) Leukosyytit [leuk0]

115) CRP [CRP0]

116) Kreatiniini [krea0]

Päivä 1

117) Hemoglobiini [Hb1]

118) Trombosyytit [tromb1]

119) Leukosyytit [leuk1]

120) CRP [CRP1]

121) Kreatiniini [krea1]

Päivä 2

122) Hemoglobiini [Hb2]

123) Trombosyytit [tromb2]

124) Leukosyytit [leuk2]

125) CRP [CRP2]

126) Kreatiniini [krea2]

Päivä 3

127) Hemoglobiini [Hb3]

128) Trombosyytit [tromb3]

129) Leukosyytit [leuk3]

130) CRP [CRP3]

131) Kreatiniini [krea3]

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Combined interventions are effective in MRSA control

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Key words: epidemic; methicillin-resistant Staphylococcus aureus (MRSA) in humans; hand hygiene; surveillance; infection control

Running title: MRSA control

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Abstract

Background: A large healthcare-associated epidemic mainly caused by one methicillin-resistant Staphylococcus aureus (MRSA) strain broke out in Pirkanmaa County, Finland, in 2001. This study describes the impact of infection control and screening practices on the epidemic.

Methods: The number of hospital-acquired (HA)-MRSA findings obtained from clinical and screening samples during the epidemic was calculated. Strains were typed by pulsed-field electrophoresis (PFGE) or spa typing. Strain type distribution was studied in relation to sample type, year of the epidemic and site of transmission. Several infection control interventions were launched stepwise and screening protocols were expanded.

Results: A total of 4118 cases were identified during 2001–2014, of which 3527 were classified as HA. One strain (spa t067) dominated in the epidemic. HA-MRSA cases decreased constantly from the year 2011. The number of new HA-MRSA cases was 57% less in the year 2014 (n=171) as compared with the year 2011 (n=399). The proportion of the epidemic strain declined significantly over the years. Screening samples comprised 71% (2439/3527) and clinical samples 29% (1034/3527) of HA-MRSA findings. The number of HA-MRSA cases found from clinical samples started to decrease when screening was expanded. An increase in hand-rub consumption was associated with a decrease in transmissions in Tampere University Hospital (TAUH).

Conclusion: Implementation of universal screening together with several other interventions is effective in containing an MRSA epidemic. The proportion of other than Pirkanmaa epidemic (PE)-

MRSA strain findings increased throughout the period, indicating the changing epidemiology of MRSA.

Keywords: Epidemic, methicillin-resistant Staphylococcus aureus (MRSA) in humans, hand hygiene, surveillance, infection control

Introduction

Overall incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) has remained low in the Nordic countries. A district-wide MRSA epidemic started in Pirkanmaa County, Finland, in August 2001, when a new epidemic MRSA strain spread rapidly in several institutions. The epidemic has continued ever since [1,2].

The importance of infection control measures to control MRSA in the healthcare setting is undeniable, but there is controversy as to the effectiveness of the different strategies [3,4]. This population- based study describes the impact of screening protocols and combined infection control interventions on the epidemic and the changing epidemiology of MRSA during the epidemic.

Material and Methods

Pirkanmaa is a county in Southern Finland (population 522 993). Primary care is provided in health centres run by the municipalities, while secondary and tertiary care are organized by the Pirkanmaa Hospital District (HD). Tampere University Hospital (TAUH) provides secondary and tertiary care with 942 beds. Secondary care hospitals (SHs) constitute 3 district hospitals with a total of 378 beds and Tampere City Hospital (249 beds). In other municipalities there are a total of 18 health centres with primary care wards. They provide mainly short-term primary care and, to a lesser extent, long-term

care. In addition, Pirkanmaa hosts 128 institutions for the long-term care of elderly people and 64 institutions for other patient groups. For our analysis, cases from health centre wards and long-term units were combined since many health centre wards also offer long-term care (long-term facilities, LTFs).

In the present study, a case was defined as a person with a new finding of MRSA cultured from either a screening or a clinical sample during August 2001 to December 2014. The MRSA database run by the HD's Infectious Disease Unit (IU) includes all MRSA findings in Pirkanmaa and contains demographic information, the type of case (i.e. healthcare-associated (HA), community-acquired or findings in healthcare workers), and the site of transmission of HA-MRSA (i.e. TAUH, SH or LTF). The type and the site of transmission were evaluated by two experienced infection control nurses by studying the patient history.

HA-MRSA cases comprised the material of the present study. At the beginning of the epidemic MRSA was defined as HA if the case had a history of in-patient admission after September 2001 in any healthcare facility (TAUH, SH or LTF) in Pirkanmaa. From the year 2006 onwards all cases who had been treated in any healthcare facility in Pirkanmaa during the previous 2 years were considered HA cases. The site of transmission (TAUH, SH or LTF) was also evaluated thoroughly: (1) if MRSA was found in a clinical sample taken within 48 h after the hospitalization, the transmission was linked to the former in-patient hospitalization (from the year 2006 only two preceding years were counted); and (2) if screening samples were negative on hospital admission but samples (clinical or screening) taken 48 h or later after the hospitalization were found to be positive, the transmission was linked to the current hospitalization. The database also contains information as to whether the sample is a screening sample or a clinical sample. The clinical samples were classified into five categories: blood, a superficial focus, a deep focus (other than blood), urine and other.

Strain typing was conducted at the National Institute of Health and Welfare, as described else- where [5]. Until 2009, pulsed field gel electrophoresis (PFGE) was used as the primary method, and strains were designated as FIN types. PFGE was substituted by spa typing in January 2009 because of its rapidity, good discrimination capacity and suitability for international comparison [6].

The Pirkanmaa epidemic has been caused mainly by one epidemic strain, FIN-16(PFGE)/t067(spa). In this context, it is termed Pirkanmaa epidemic (PE)-MRSA. Based on our previous analysis, we know that there is 97% concordance between FIN-16 and t067, and thus both are classified as PE-MRSA [1,6]. All other PFGE types found before 2009 were classified into one group, named non-FIN-16. The first positive sample from each patient was included in the analysis.

The screening protocol for MRSA up to May 2008 recommended targeted screening, i.e. screening of each patient admitted to TAUH if they had been treated in specified institutions with known existence of MRSA (screening period I). Between June 2008 and September 2011, patients were screened if they had a history of in-patient care in any healthcare facility in the county after 2001 (screening period II). After September 2011, all patients admitted to the HD's wards were screened for MRSA regardless of their history of care (screening period III). This universal screening has also been recommended to health centres and long-term care institutions. Only women in labour without an in-patient history and children are excluded. Screening samples were taken from throat, nostrils, catheters, drainage tube sites and skin lesions. Health care workers were not screened unless they fulfilled other screening criteria.

Standard precautions are recommended in the care of all patients, and if they were found to be MRSA-positive they were treated with contact precautions (treated in a single room, and use of gowns, gloves and masks when in close contact with a patient). In addition to expanding screening recommendations during the epidemic, other improvements in infection control have been implemented stepwise during

the epidemic (Table I). At the end of 2011, a working group for the MRSA epidemic was set up by the Board of Pirkanmaa HD.The members of the MRSA Working Group are the medical director, heads of different medical divisions, personnel manager of TAUH and three infectious disease specialists from the IU. The Working Group supervises the actions and surveillance in the Pirkanmaa MRSA epidemic. A summary of the current situation is reported monthly to the Working Group. Every case is also reported to the ward that was the probable site of transmission and analysed with the link nurse and ward personnel. Since 2011 infection control compliance has been promoted by regular visits by the IU's infection control nurses in TAUH wards and in LTFs. In TAUH, SH and LTFs in Pirkanmaa, a link nurse network has been reinforced from the beginning of 2012. Their work is supervised by the IU's infectious disease specialists and infection control nurses, and the SHs' infection control nurses.

The central idea of the MRSA Working Group and the IU is to promote standard precautions in the treatment of all patients, to stress the importance of good hand hygiene, to extensively recognize new MRSA cases and to identify healthcare units where urgent actions for improvement of infection control practices are needed.

To evaluate the effectiveness of hand hygiene promotion in TAUH, the use of alcohol hand-rub was calculated using the amount of hand-rub ordered (litres) in each department in TAUH, divided by the number of patient days. This was compared to the number of new MRSA cases in TAUH in relation to the number of patient days.

An SPSS package (version 22) was used for statistical analyses and a two-sided p value<0.05 was taken as a cut-off for statistical significance. Categorical data were analysed by the x^2-test or Fisher's exact test when appropriate.

Results

During the epidemic, a total of 4118 new MRSA cases were identified in Pirkanmaa, of which 3527 (87%) were classified as HA and constituted the cases in the present study. There were 39 (1%) cases in healthcare workers and 53 cases (1%) from other areas of Finland. In all, 436 cases (11%) cases were community acquired. The remaining 62 (2%) cases were transmissions from abroad.

HA-MRSA cases decreased constantly from the year 2011.The number of new HA-MRSA cases was 57% less in the year 2014 (n=171) as compared with the year 2011 (n = 399) (Figures 1 and 2).

Until January 2009, new HA-MRSA cases comprised 17 different FIN types (n = 1729). Altogether 91% of cases (1574/1729) represented PE-MRSA. Since the adoption of spa typing in January 2009, 98 different spa types have been found (n = 1798), and 67% (1204/1798) of cases were of PE-MRSA. Other most prevalent spa types were t008 (7%), t1012 (5%), t172 (4%) and t032 (3%). During the whole epidemic, 79% (2778/3527) of all cases have been PE-MRSA.

As the screening recommendations have changed, the average number of screening samples taken each year has increased. The number of screening samples taken yearly during screening periods I, II and III were approximately 48 000, 132 000 and 228 000, respectively. Since several screening samples are taken from each patient, the number of screened patients is actually much smaller.

Screening samples comprised 71% (2439/3527) and clinical samples 29% (1034/3527) of HA-MRSA findings. Of the clinical samples, 71% (734/1034) were from superficial focus, 21% (216/1034) from urine samples, 3% (35/1034) from blood cultures, 3% (35/1034) cultures from other deep focus and 1% (14/1034) from undefined focus. The proportion of cases found in clinical samples decreased in relation to screening periods. During screening period I, 46% (676/1469) of new cases were found in clinical samples, and in screening periods II and III the proportion decreased to 19% (238/1235) and 15% (120/823), respectively (p = 0.001 for comparisons screening period I vs screening period II and

screening period I vs screening period III).

The number of new HA-MRSA cases found through clinical or screening samples each year during the epidemic and their spa type distribution is presented in Figure 1 in relation to the infection control interventions, shown in Table I. The number of new HA-MRSA cases found through clinical or screening samples has been decreasing constantly from the year 2011. The proportion of PE-MRSA in clinical samples has decreased from its peak of 99% (79/80) in 2005 to 45% (13/29) in 2013 (p = 0.001). In screening samples the proportion of PE-MRSA decreased from 98% (120/123) in 2005 to 45% (69/155) in 2014 (p = 0.001).

The majority of new HA-MRSA cases throughout the whole epidemic have been encountered in LTFs and have represented PE- MRSA. Throughout screening period II the number of new cases remained high both in LTFs and in hospitals (TAUH and SH). During screening period III the number of new cases started to decrease both in LTFs and in hospitals (Figure 2). The proportion of PE-MRSA has decreased at all levels of healthcare already from screening period II.

Figure 3 presents the correlation between alcohol hand-rub consumption in TAUH and the number of new HA-MRSA cases where the site of transmission was TAUH in relation to the number of patient days in TAUH. Hand-rub consumption increased 2.5- fold during the study period.

Discussion

The MRSA incidence has remained low in Finland, although a slight gradual increase has occurred within recent decades. On such a scale, the Pirkanmaa epidemic is large and exceptional and has resulted in MRSA incidence far higher than in other regions in Finland. During the years 2012–2014 the Pirkanmaa epidemic has been successfully controlled due to combined interventions.

In the early stage of the epidemic, a decrease of HA-MRSA cases found through clinical samples was seen at the beginning of screening period II (targeted screening). This decrease may have been influenced by an interim infection control promotion project in 2009 (Table I, Figure 1). HA-MRSA cases found through clinical samples is a stable parameter over time, thus this reduction emphasizes the success in MRSA control. However, after completing that project, the HA-MRSA figures started to rise again and therefore universal screening was launched in 2011. HA-MRSA cases found through screening samples started to decline at the beginning of the year 2012. A constantly decreasing number of HA-MRSA in both clinical and screening samples from the year 2011 onwards is probably due to universal screening and several other permanent interventions launched as shown in Table I.

The findings of two recent studies have indicated that targeted screening can miss a substantial proportion of MRSA carriers [7,8]. Otter and co-workers estimated that targeted screening would fail to identify about 45% of carriers as compared with universal screening, which may have implications for MRSA control [8]. In the present study, the universal screening has led to the recognition of a wider spectrum of MRSA strain types.

PE-MRSA has been the dominating strain; however, its proportion has gradually decreased. This may result partly from changes in screening protocols, but might also be influenced by the natural strain dynamics reported earlier in the UK [9,10]. The latter is supported by the fact that the proportion of PE-MRSA also decreased significantly in clinical samples. The proportion of other spa types, especially t008 and t1012, is increasing in HA-MRSA cases. The expanding genetic diversity of MRSA strain types has been noted globally [11].

Active surveillance and early recognition of MRSA cases are vital for the implementation of contact precautions, but also reveal patients at risk of MRSA infection, facilitating correct choice of

prophylactic or empiric treatment. In the early years of the epidemic new MRSA findings were recovered mainly from clinical samples, but after the screening criteria were expanded most findings were from screening samples. MRSA colonization usually precedes clinical infection, which suggests that cases were previously insufficiently detected [12,13]. Even with universal screening, 15% of new MRSA findings were recovered from clinical samples. In comparison, a recent Swedish study reported that 36% of new MRSA findings were recovered from clinical samples [14]. Lee and associates reported that 90% of MRSA cases would be missed if only clinical cultures were used [15].

In addition to expanding screening recommendations during the epidemic, other interventions to improve infection control have also been employed, as described in Material and Methods. Throughout screening period III infection control compliance has been promoted by regular infection control nurse visits in wards. Link nurses are a part of this work and their contribution is expanding. The setting up of the MRSA Working Group by the Board of HD shows the commitment of the leadership and this is of utmost importance.

Hand-rub consumption more than doubled from 2005 to 2014 (420–1080 L/10 000 patient days) in TAUH. After 2010, there is an inverse relation between alcohol hand-rub consumption and MRSA transmissions in TAUH. Doubling the hand-hand rub consumption was related to about 67% decrease of new HA-MRSA cases in TAUH. Earlier studies would imply that hand hygiene is the most successful policy in MRSA control [16,17], but some reports [18] have suggested that a 60% increase in hand hygiene compliance reduces the MRSA prevalence by only 20%. Some studies have predicted that only an improvement in hand hygiene from a very poor level has any significant impact on *S. aureus* preva- lence [19], whereas improvement above levels of 40% compliance has only a small effect. In the present study the number of MRSA cases started to decrease when hand-rub consumption exceeded 925 L/10 000 patient days. We may hypothesize that consumption must exceed a certain

critical level before its effect on MRSA rates is seen. However, universal screening was also implemented when the decrease in MRSA cases started. A recent study reported that neither enhanced hand hygiene nor universal screening with contact precautions and decolonization of cases had any significant effect on MRSA rates alone, but when combined, MRSA rates decreased by 12% in clinical samples [15]. In the present study the decrease in HA-MRSA cases found in clinical samples is much more remarkable: 137 cases in clinical samples in the year 2008 as compared with 16 cases in the year 2014.

A major strength of this study is the population-based design. All the samples in the district were examined in a single central laboratory and strain typing was conducted in a national centre. All health-care facilities in the area are advised to follow alignment to the district's MRSA guidelines. Therefore, the obtained data can be regarded as a good representative of the situation among the population in the area. The incidence of MRSA was very low at the beginning of the outbreak. This gave us an opportunity to observe the impact of measures during different stages of the epidemic. A limitation of this study is that it is observational and many interventions have been launched stepwise and simultaneously. The effect of a single intervention on HA-MRSA figures cannot be assessed. The main goal of the MRSA Working Group and IU has been to contain the epidemic. Therefore, several elements of infection control were involved simultaneously when changes to guidance were made.

Several infection control measures to reduce transmission of MRSA have been implemented step- wise during the epidemic. This, together with the universal screening policy, has led to a significant reduction in new HA-MRSA cases and the change in the strain distribution.

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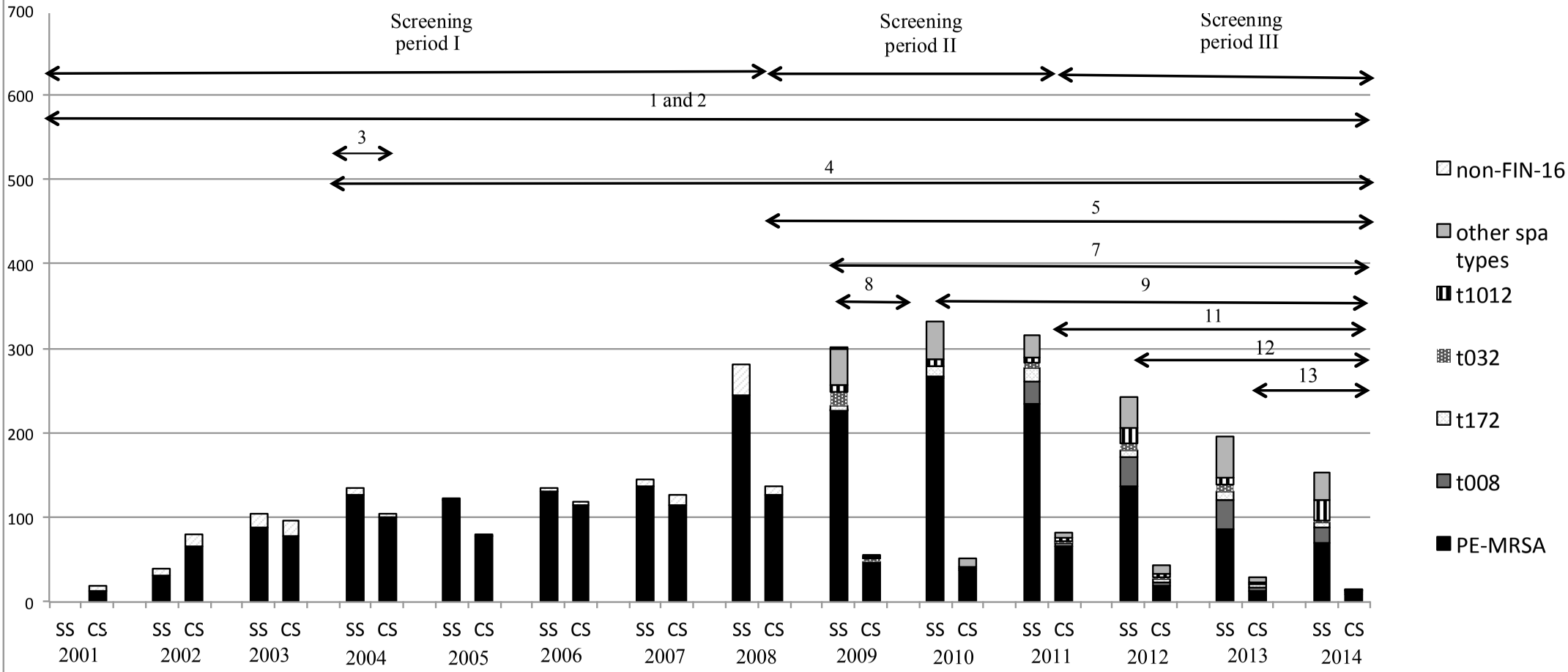
Table 1. MRSA control interventions (other than screening) launched during the epidemic	
Intervention	Time
1) Emphasizing the use of standard precautions in all care	Throughout the epidemic
2) Emphasizing the use of contact precautions in care of MRSA carriers in hospitals	Throughout the epidemic
3) Infection control promotion project in LTFs	2004
4) Link nurse network established in LTFs	2004-
5) MRSA cohort in TAUH	2008-
6) MRSA dialysis	2009-
7) Infection control promotion project in Tampere University Hospital	2009
8) Three mobile infection control nurses were recruited to visit long-term units regularly	2010-
9) Working group for MRSA epidemic	2011-
10) Reinforcement of link nurse network in TAUH	2012-
11) MRSA decolonization for selected patient groups in TAUH	2013-

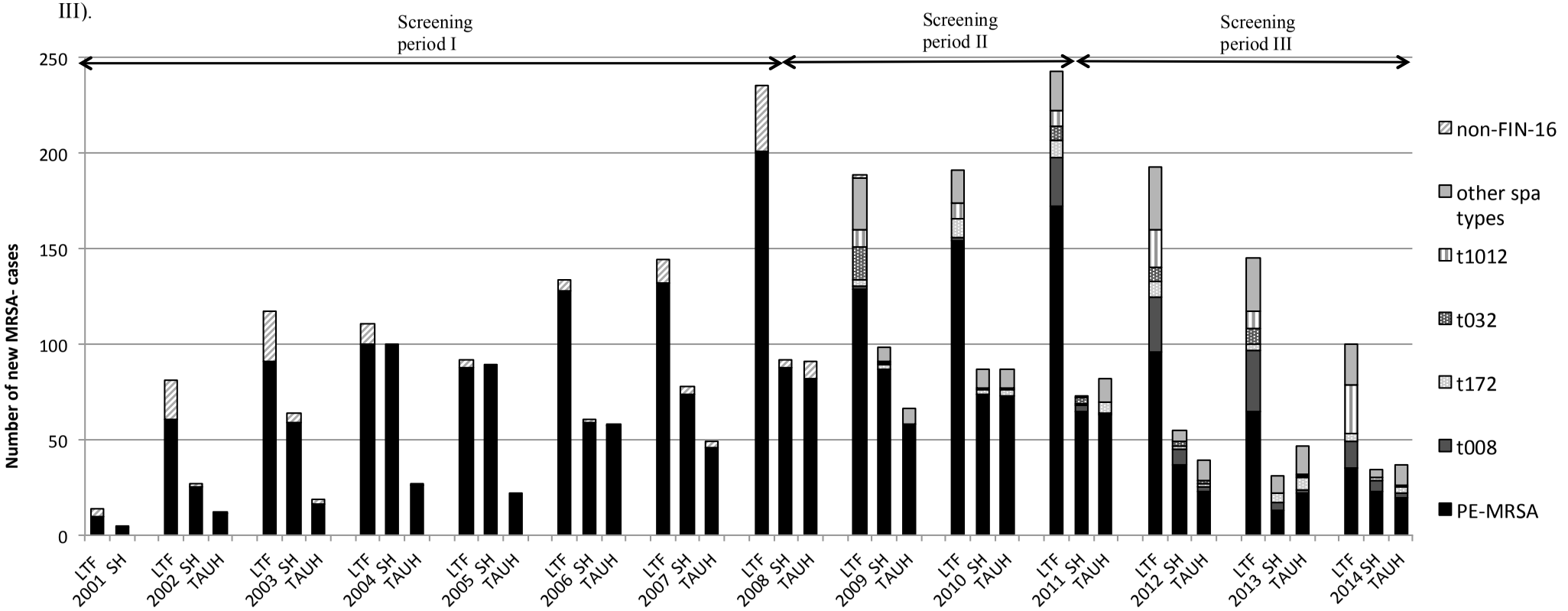
MRSA; methicillin-resistant *Staphylococcus aureus*, TAUH; Tampere University Hospital, LTF; long-term facilities

Figure 1. Number on HA-MRSA cases recovered from clinical samples and screening samples and their strain type distribution in relation to year of transmission. Black arrows indicate the screening period (I, II and III) and other interventions. The interventions are numbered as given in table 1.



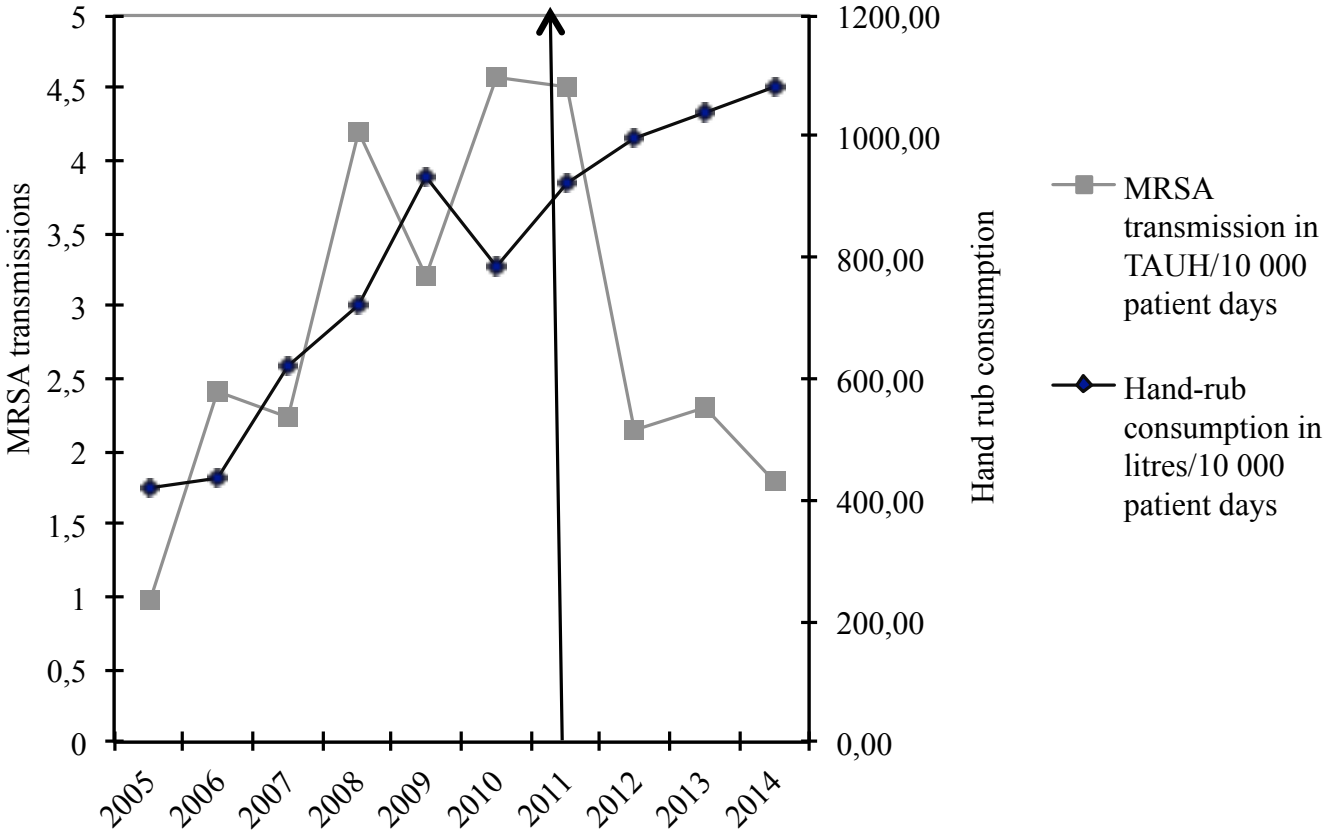
MRSA: methicillin-resistant *Staphylococcus aureus*; SS: Screening sample; CS: clinical sample.

Figure 2. Distribution of different HA-MRSA strain types in relation to year and location of transmission. Black arrows indicate the screening periods (I, II or III).



MRSA: methicillin-resistant *Staphylococcus aureus*; LTF: long-term facilities; SH: secondary hospitals; TAUH: Tampere University Hospital.

Figure 3. Relation of alcohol hand-rub consumption in litres per 10 000 patient days and number of MRSA transmissions per 10 000 patient days in Tampere University Hospital between 2005 and 2014. Black arrow indicates the beginning of universal screening.



MRSA: methicillin-resistant *Staphylococcus aureus*; TAUH: Tampere University Hospital

Original article

Comparison of outcome and clinical characteristics of bacteremia caused by methicillin resistant, penicillin resistant and penicillin susceptible *Staphylococcus aureus* strains

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Running title: Antibiotic resistance in *S. aureus* bacteremia

Key words: MRSA, PRSA, PSSA, *Staphylococcus aureus*, Bacteremia

Conflict of Interest: The authors declare that they have no conflicts of interest.

Abstract

Background: The aim of this study was to assess the association of methicillin resistance and penicillinase production with clinical characteristics and outcome of Staphylococcus aureus bacteremia. Methods: For 126 patients with methicillin resistant Staphylococcus aureus (MRSA) bacteremia, 378 age and gender matched controls with methicillin susceptible Staphylococcus aureus (MSSA) bacteremia were selected. Of controls, 126 had bacteremia caused by penicillin susceptible strains (PSSA) and 252 by penicillinase producing strains (PRSA). Underlying diseases, clinical course and mortality were retrospectively assessed. Results: Patients with MRSA bacteremia were more often smokers than patients with MSSA bacteremia (OR 2.34, 95% CI 1.27-4.32). MRSA bacteremia was more often healthcare-associated (OR 4.23, 95% CI 2.47-7.24), associated with central venous catheters (OR 2.09, 95% CI 1.27-3.47), glucocorticoid therapy (OR 1.82, 95% CI 1.12-2.93) and prior surgery (OR 2.32, 95% CI 1.43-3.76). Patients with MRSA bacteremia received appropriate empiric antibiotic (31%) less often than controls (98%). Mortality within 28 days was higher in MRSA bacteremia (26,8%) than in MSSA bacteremia (15.5%) (OR 2.00, 95% CI 1.20-3.34), PRSA bacteremia (17.0%) (OR 1.79 95%, CI 1.04-3.09) or PSSA bacteremia (12,5%) (OR 2.56 95%, CI 1.27-5.15). The difference remained after adjusting for underlying diseases and foci. There was no significant difference in clinical course between PRSA and PSSA bacteremias. Conclusions: MRSA bacteremia was associated with poorer outcome than either PRSA or PSSA bacteremia. We corroborated several risk factors found in previous studies.

Introduction

Differences in clinical course and mortality between bacteremias caused by methicillin resistant (MRSA) and methicillin susceptible (MSSA) *Staphylococcus aureus* have been reported [1-3]. MRSA has been associated with higher mortality in most but not all studies [2-5]. Higher mortality

may result from higher background morbidity, older age and delay in appropriate antimicrobial therapy in MRSA bacteremia patients [2,3,6]. Less attention has been paid to differences between bacteremias caused by penicillin susceptible (PSSA) strains and penicillinase producing strains (PRSA). In Europe, from 13 to 35 per cent [7] of MSSA isolates remain susceptible to penicillin and some recent studies have suggested that the proportion of penicillin susceptible strains might even be rising [8]. Penicillin has clinical advantages over cefuroxime in treatment of PSSA bacteremias [9], which emphasizes the importance of distinction of PSSA and PRSA strains in clinical practise. There are no studies comparing MRSA bacteremias separately to PSSA or PRSA bacteremias. To optimize treatment, it is important to detect potential differences in clinical characteristics between *S. aureus* bacteremias caused by strains with different antimicrobial susceptibility.

The aim of this study was to assess the difference in clinical characteristics, severity and outcome of *S. aureus* bacteremia caused by MRSA, PRSA and PSSA strains.

Methods

The study was conducted in Pirkanmaa County in Southern Finland (population 524 711). All MRSA bacteremia episodes diagnosed in the county from 2002-2013 were included (n=126). Information on bacteremia episodes was obtained from the central laboratory (Fimlab), which analyses all blood cultures in the county. MRSA strains were identified using Clinical and Laboratory Standards Institute (CLSI) guidelines until 2011 and since then using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. The presence of the *mecA* gene was tested using GenoType®MRSA (Hain Lifescience) [10]. A case was defined as a patient with at least one blood culture positive for *S. aureus* together with clinical signs and symptoms of an infection. If a case had recurrent bacteremias, episodes were concidered separate if

they were noticed at least 30 days apart. For each MRSA case (n=126), three gender and age matched controls with MSSA bacteremia were selected (n=378). Of each three controls, one had bacteremia caused by PSSA and two by PRSA. Recurrent episodes and their controls were excluded from the mortality analysis.

Clinical data was retrospectively recovered from hospital records. Clinical data included underlying diseases, immunocompromising medication, smoking, body mass index (BMI), indwelling devices, traumas, central venous catheters (CVCs), surgery and other invasive procedures. Intensive care unit (ICU) admission, acute renal replacement therapy and invasive ventilation were recorded. A complete list of examined items is presented in an Appendix. The foci of infection were evaluated. A single patient may have had various foci simultaneously. Endocarditis was defined using the modified Duke criteria [11] . Patients with CVC were considered to have a CVC-associated bacteremia regardless of other foci [12]. If there was purulent secretion or redness around a catheter, the patient was considered to have a skin and soft tissue infection (SSTI) as well. Appropriateness of empiric treatment (defined by *in vitro* susceptibility) was recorded. Mortality on day 28 (d28) after the positive blood culture was used as an indicator of outcome. Mortality data was retrieved from electronic hospital records. Information on possible date of death is updated to hospital records from National Population Registry weekly, and was available for all cases. Bacteremia was classified as community aqcuired (CA) or healthcare-associated (HA) by studying the patient´s medical history. HA bacteremia was defined according to the Finnish national surveillance study [13,14]; an infection was defined HA if the symptoms started more than 48 hours after hospital admission or the bacteremia was related to surgical operation within the past 30 days or other invasive procedure within the last 10 days. Patients on chronic renal replacement therapy (hemo- or peritoneal dialysis) or neutropenic due to treatment of malignancy were classified as having a HA bacteremia.

A SPSS package (version 22) and STATA (version 13.0, Statacorp, Texas, USA) were used for statistical analyses. Conditional logistic regression was used for analysis of underlying diseases and foci. Results are presented in odds ratios (OR) with 95% confidence intervals (95% CI). Chi square test was used for analysis of categorical data. Logistic regression was used for univariate analysis of variables associated with d28 mortality. Only variables significant in univariate analysis were entered into the multivariable model.

The study was approved by the Ethical Review board of the Pirkanmaa Health District. Informed consent was not required because analysed data did not contain identifying information.

Results

A comparison of the underlying conditions of MRSA, MSSA, PSRA and PSSA bacteremia patients are presented in Table I. Median age of MRSA bacteremia patients was 74 years and of MSSA bacteremia patients 73 years (both PRSA and PSSA). Median body mass index (BMI) was 26 kg/m^2 in MRSA, MSSA and PRSA patients. Median BMI in PSSA patients was 27 kg/m^2. BMI data was missing from 14% (69/504) of cases and smoking data was missing from 24% (121/504) of cases.

MRSA cases were more often smokers, had previous surgery, glucocorticoid therapy and were classified as having a HA bacteremia than MSSA controls. Similar risk factors were observed in comparison to PRSA controls. In comparison of MRSA cases to PSSA controls, only previous surgery and HA infection were significant risk factors. There was no significant difference in any underlying conditions between PRSA and PSSA bacteremias.

In the analysis of the foci of infection (Table II), MRSA bacteremia was more often associated with CVC than MSSA bacteremia.The most common focus was SSTI in both MRSA and in MSSA infections. MRSA bacteremia was more often associated with SSTI than PRSA bacteremia but not than PSSA bacteremia. The focus remained unknown equally often in MRSA and MSSA bacteremias. There was no significant difference in foci between PRSA and PSSA bacteremias. Focus data was missing from 4% (20/504) of cases.

Altogether 31% (37/118) of MRSA cases and 98% (349/358) of MSSA controls received adequate empiric antibiotics (Table III). Altogether 67% (85/126) of MRSA cases were previously known carriers of MRSA and 40% (34/85) of them received appropriate empiric antibiotic. Data on empiric antibiotic treatment was missing from 6% (28/504) of cases.

Acute renal replacement therapy was needed more often in MRSA cases compared to MSSA and to PRSA (Table III). However, ICU admittance or invasive ventilation did not differ between groups (Table III).

Eleven MRSA cases and three MSSA controls had recurrent bacteremias. Only the first episode was included in the mortality analysis.

Of all bacteremias, 64% (317/504) were HA. Data on HA or CA was missing from six cases. d28 mortality was 18% both in HA (51/277) and CA cases (30/165) (OR 1.02, 95%, CI 0.62-1.67).

Factors associated with d28 mortality are presented in Table IV. Methicillin resistance, age, cardiac disease, liver cirrhosis and previous glucocorticoid therapy were significantly associated with d28

mortality in multivariable analysis. Appropriate antimicrobial therapy was a protective factor for d28 mortality in univariate analysis but not in multivariable analysis.

Pneumonia as a focus was associated with higher d28 mortality (OR 2.51, 95% CI 1.42 to 4.44) but SSTI (OR 0.42, 95% CI 0.24 to 0.74) and osteomyelitis or spondylodiscitis (OR 0.32, 95% CI 0.11 to 0.90) with lower mortality. An unknow focus was associated with higher mortality (OR 1.78, 95 % CI 0.98-3.23). No other associations between infection foci and d28 mortality were found (deep abscess, native arthritis, CVC-associated bacteremia, other infected foreign body, endocarditis and mediastinitis).

Table V presents a comparison of d28 mortality in MRSA, MSSA, PRSA and PSSA bacteremias adjusted for factors associated with d28 mortality in univariate analysis (Table 4 and foci). d28 mortality of MRSA cases who received effective antibiotic was 31% (10/32) and in those who did not receive effective antibiotic 25% (18/73). The difference was not significant (OR 1.39, 95% CI 0.56-3.48)

Discussion

MRSA as a cause of bacteremia was associated with higher d28 mortality than MSSA. This association remained significant in multivariable analyses adjusted for underlying diseases and foci and was more pronounced when MRSA was compared to PSSA than when compared to PRSA. d28 mortality did not differ significantly between PRSA and PSSA bacteremias which is consistent with findings in a study by Cheng et al [15]. However, the resistance profile per se may not be the only factor influencing the prognosis. There are probably a number of virulence factors determining the outcome as well.

Acute renal replacement therapy was needed more often in MRSA bacteremia compared to MSSA, possibly reflecting more severe disease. Although MRSA cases were of the same age as their matched controls, patients with MRSA probably had more severe underlying diseases since there were significant differences in the use of CVCs, previous surgery and glucocorticoid therapy.

There is substantial variation in the definition of HA bacteremia. In this study, Finnish national surveillance definition was applied [13,14] which slightly differs from the definition used by CDC [16]. The proportion of HA bacteremias was higher in MRSA cases reflecting the higher frequency of CVCs and previous surgery. Healthcare-association was not a significant risk factor for d28 mortality, which is consistent with several studies [17-19]. Due to a higher proportion of HA bacteremias in MRSA cases, these could be prevented by improving infection control strategies [20].

We found that current smoking was significantly more common among MRSA cases than among controls infected by MSSA. It is thought that *S. aureus* colonization precedes *S. aureus* bacteremia. Interestingly, smoking has been associated with *S. aureus* nasal carriage [21] and second-hand smoke exposure in infants with cystic fibrosis has been associated with MRSA colonization [22]. Cigarette smoke increases MRSA adherence to and invasion of epithelial cells [23] and increases *S. aureus* biofilm formation [24]. Some studies have reported that smoking might be associated with MRSA infections [25-27]. There are no earlier reports of an association between smoking and MRSA bacteremia.

Glucocorticoid therapy was more common in patients with MRSA bacteremia than in patients with MSSA bacteremia. In an earlier study glucocorticoid therapy was associated with HA bacteremias

caused by *S. aureus* but the association between glucocorticoid therapy and MRSA bacteremia was not reported in that study [28]. Another study [29] reported that patients with CA-MRSA infection or colonisation were more often on glucocorticoid therapy than age matched controls without CA-MRSA infection or colonisation. In patients with systemic lupus erythematosus glucocorticoid therapy is a risk factor for infections caused by multidrug resistant bacteria, including MRSA [30]. Most previous studies do not analyse association of a glucocorticoid therapy to MRSA infections separately, as only association of heterogeneously defined immunosuppressive status and infections caused by multidrug resistant bacteria have been addressed.

The focus of infection was unknown in 17% of the study population and there were no differences between MRSA, PRSA and PSSA cases. A similar percentage (19%) was reported in a prospective study by Jacobsson et al [19]. Several studies [19,31] have shown that there is a strong association between death and an unidentified infective focus. Our results are in line with previous studies. The most common focus was SSTI (39%). In a pooled analysis of *S. aureus* bacteremia studies performed by Kaasch et al [31], the most common foci were central or peripheral catheters (28%). The proportion of CVC-associated *S. aureus* bacteremias was 19% (93/479) in our study, equal to the study by Kaasch et al [31]. SSTI was associated with better prognosis as was spondylodiscitis or osteomyelitis. This may be related to the specific symptoms from these foci, which may lead to earlier diagnosis and treatment. However, in a study by Jacobsson et al [19], SSTI and vertebral osteomyelitis had a neutral effect on mortality, but osteomyelitis was related to a better outcome. Pneumonia was associated with a poorer outcome in our study, which is consistent with previous studies [3,17,18].

In a study by Kaasch et al [31], endocarditis was diagnosed in 8% of cases and an echocardiogram was performed in 57% of the patients. In our study, 4 % of MRSA bacteremia and 7% of MSSA

cases had endocarditis. Either transthoracic echocardiography (TTE) or transesophageal (TEE) echocardiography or both were performed less often in MRSA cases (28 %) than MSSA cases (47 %). This may be due to the fact that in MRSA cases removable foci (CVCs) were more commonly found. There was no difference between MRSA and MSSA bacteremias in the percentage of endocarditis found on echocardiography. However, it is possible that some MRSA endocarditis were missed due to not performing echocardiographies although also in a study by Yaw et al, fewer patients with MRSA bacteremia (5%) had endocarditis diagnosed compared to MSSA bacteremia patients (11 %)[2].

Contrary to MSSA cases, the majority of MRSA cases did not receive appropriate empiric antibiotic. In univariate analysis including both MRSA and MSSA cases, appropriate empiric treatment was a protective factor for death within 28 days but was not significant in multivariable analysis adjusted for underlying conditions. In the studies by Gasch et al [17], and by Yaw et al [2], 66% and 34% of MRSA bacteremia patients received appropriate empiric therapy. In several studies, inappropriate empiric therapy was the most important predictor of death in MRSA bacteremia [17,32,33] However, Kaash et al [34] and Jacobsson et al [19] showed that in patients with *S. aureus* bacteremia early appropriate antimicrobial therapy may not improve survival. In our study, appropriate empiric therapy did not affect significantly d28 mortality in MRSA cases. However, this might be due to small sample size.

There are some limitations of this study. It is of retrospective design and relies on accuracy of written hospital records. Also some data is missing. The study population is relatively small. Despite the matching of MRSA and MSSA cases on age and gender, we may have missed confounding factors affecting the clinical course of *S. aureus* bacteremia.

In conclusion, the present study shows that d28 mortality is higher in MRSA bacteremia compared to bacteremia caused by MSSA, PSSA or PRSA. There were no differences in clinical characteristics or d28 mortality between PSSA and PRSA bacteremias. Smoking and glucocorticoid therapy were associated with MRSA bacteremia in addition to more traditional associations, such as CVCs, surgery and hospital acquisition of infection.

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Table I. Underlying conditions of patients with *Staphylococcus aureus* bacteremia. Unadjusted odds ratios are calculated using conditional logistic regression for comparisons MRSA vs MSSA, MRSA vs PRSA, MRSA vs PSSA. Percentages are computed as the per cent of all non-missing values.

	MRSA n (%)	MSSA n (%)	OR	95% CI	PRSA n (%)	OR	95% CI	PSSA n (%)	OR	95% CI
Male	69 (55)	207 (55)	1.00	0.65-1.50	138 (55)	1.00	0.65-1.53	69 (55)	1.00	0.61-1.64
Current smoking[a]	31 (37)	63 (21)	2.34	1.27-4.32	41 (20)	2.23	1.20-4.17	22 (23)	1.88	0.80-4.42
Cardiac disease	71 (56)	204 (54)	1.12	0.72-1.75	135 (54)	1.14	0.71-1.84	69 (55)	1.08	0.62-1.89
Chronic kidney disease	44 (35)	111 (30)	1.26	0.82-1.95	66 (26)	1.49	0.93-2.39	45 (36)	0.93	0.54-1.58
Chronic obstructive pulmonary disease or asthma	26 (21)	55 (15)	1.52	0.91-2.53	38 (15)	1.46	0.84-2.53	17 (14)	1.64	0.85-3.19
Chronic or traumatic ulcer	40 (32)	108 (29)	1.17	0.75-1.81	81 (32)	0.98	0.62-1.56	27 (21)	1.68	0.95-2.97
Dermatitis	17 (14)	60 (16)	0.83	0.47-1.48	44 (17)	0.74	0.41-1.35	16 (13)	1.08	0.51-2.29
Diabetes (type 1 or 2)	45 (36)	112 (30)	1.34	0.86-2.06	80 (32)	1.22	0.76-1.98	32 (25)	1.56	0.93-2.64
Liver cirrhosis	10 (8)	28 (8)	1.08	0.50-2.34	18 (7)	1.13	0.50-2.56	10 (8)	1	0.37-2.52
Foreign body[b]	44 (35)	100 (27)	1.53	0.98-2.40	64 (25)	1.68	1.02-2.76	36 (27)	1.33	0.79-2.26
Chronic renal replacement therapy (HD or PD)	12 (10)	49 (13)	0.71	0.36-1.37	34 (13)	0.66	0.32-1.35	15 (12)	0.77	0.34-1.75
Glucocorticoid therapy ≥5 mg/day (within previous month)	38 (30)	74 (20)	1.82	1.12-2.93	44 (18)	2.05	1.22-3.42	30 (24)	1.41	0.76-2.62
Immunosuppressive therapy	13 (10)	37 (10)	1.07	0.53-2.15	23 (9)	1.18	0.53-2.60	14 (11)	0.92	0.04-2.08
Prior surgery	38 (30)	61 (16)	2.32	1.43-3.76	42 (16)	2.20	1.31-3.66	19 (15)	2.46	1.29-4.69
Other invasive procedure	51 (41)	130 (35)	1.28	0.85-1.94	85 (34)	1.31	0.84-2.03	45 (36)	1.26	0.76-2.09
Healthcare-associated bacteremia	105 (84)	212 (57)	4.23	2.47-7.24	139 (55)	4.16	2.40-7.22	73 (59)	4.56	2.21-9.37

[a] Smoking data was missing from 121 (24%) cases

[b] Articular prosthesis n=81, vascular prosthesis n=22, pacemakers n=26, osteosynthesis material=27

MRSA, Methicillin resistant *Staphylococcus aureus*; MSSA, Methicillin susceptible *Staphylococcus aureus*; PRSA, penicillin resistant *Staphylococcus aureus*; PSSA, penicillin susceptible *Staphylococcus aureus*; OD, odds ratio; CI, confidence interval.

Table II. Focus in *Staphylococcus aureus* bacteremia. Unadjusted odds ratios are calculated using conditional logistic regression for comparisons MRSA vs MSSA, MRSA vs PRSA, MRSA vs PSSA. Percentages are computed as the per cent of all non-missing values.

Focus	MRSA n (%)	MSSA n (%)	OR	95% CI	PRSA n (%)	OR	95% CI	PSSA n (%)	OR	95% CI
Pneumonia	24 (20)	57 (16)	1.32	0.78-2.24	34 (14)	1.45	0.82-2.57	23 (19)	1.12	0.58-2.15
Skin or soft tissue infection	56 (46)	131 (36)	1.52	0.99-2.33	83 (35)	1.70	1.07-2.69	48 (40)	1.23	0.73-2.06
Deep abscess	8 (7)	29 (8)	0.79	0.35-1.76	17 (7)	0.88	0.36-2.12	12 (9)	0.67	0.27-1.63
Native arthritis	4 (3)	23 (6)	0.52	0.18-1.54	17 (7)	0.46	0.15-1.38	6 (5)	0.50	0.09-2.73
Central venous catheter	35 (29)	59 (17)	2.10	1.27-3.47	37 (16)	2.21	1.28-3.82	22 (18)	2.00	1.03-3.89
Infected foreign body[a]	15 (12)	36 (10)	1.39	0.76-2.65	23 (10)	1.45	0.72-2.91	13 (11)	1.27	0.58-2.80
Endocarditis	5 (4)	25 (7)	0.59	0.22-1.59	18 (7)	0.55	0.20-1.49	7 (6)	0.67	0.19-2.36
Spodylodiscitis or osteomyelitis	11 (9)	48 (13)	0.66	0.33-1.31	31 (12)	0.69	0.33-1.41	17 (13)	0.60	0.26-1.37
Mediastinitis	1 (1)	17 (5)	0.17	0.02-1.30	10 (4)	0.20	0.03-1.56	7 (6)	0.14	0.02-1.16
Unknown focus	19 (16)	65 (18)	0.83	0.48-1.42	46 (19)	0.77	0.44-1.35	19 (16)	1	0.52-1.92

[a]Infected foreign bodies: 10 vascular prosthesis, 9 osteosynthesis material, 7 pace makers, 25 articular prosthesis

MRSA, Methicillin resistant *Staphylococcus aureus*; MSSA, Methicillin susceptible *Staphylococcus aureus*; PRSA, penicillin resistant *Staphylococcus aureus*; PSSA, penicillin susceptible *Staphylococcus aureus*; OD, odds ratio; CI, confidence interval.

Table III. Treatment of patients with *Staphylococcus aureus* bacteremia. P-values are calculated using Chi square test for comparisons MRSA vs MSSA, MRSA vs PRSA, MRSA vs PSSA. Percentages are computed as the per cent of all non-missing values.

	MRSA n (%)	MSSA n (%)	p-value MRSA vs MSSA	PRSA n (%)	p-value MRSA vs PRSA	PSSA n (%)	p-value MRSA vs PSSA
Empiric antibiotic effective	37 (31)	349 (98)	<0.001	235 (98)	<0.001	114 (97)	<0.001
Echocardiogram performed	34 (28)	170 (47)	<0.001	111 (46)	0.001	59 (49)	0.001
ICU treatment	15 (12)	51 (14)	0.633	38 (15)	0.436	13 (10)	0.842
Acute renal replacement therapy	8 (6)	4 (1)	0.001	2 (1)	0.003	2 (2)	0.102
Invasive ventilation	13 (11)	28 (8)	0.297	20 (8)	0.442	8 (7)	0.362

MRSA, Methicillin resistant *Staphylococcus aureus*; MSSA, Methicillin susceptible *Staphylococcus aureus*; PRSA, penicillin resistant *Staphylococcus aureus*; PSSA, penicillin susceptible *Staphylococcus aureus*

Table IV. Logistic regression analysis of factors associated with d28 mortality in patients with *Staphylococcus aureus* bacteremia. Variables that were either statistically significant in univariate analysis or considered clinically important were entered to multivariable model. Of recurrent episodes only the first episode was included in the analysis.

		Univariate		Multivariable	
	n	OR	95% CI	OR	95% CI
Methicillin resistance	112/448	2.00	1.20-3.34	2.34	1.02-5.37
Age (years)		1.05	1.02-1.07	1.05	1.02-1.08
Male gender	244/448	0.85	0.53-1.38		
BMI		1.01	0.97-1.05		
Smoking	83/339	0.91	0.45-1.84		
Cardiac disease	240/448	3.27	1.90-5.64	1.86	1.00-3.44
Chronic kidney disease	136/444	1.63	0.99-2.68	1.21	0.69-2.11
Chronic obstructive pulmonary disease or asthma	72/448	1.09	0.58-2.07	0.77	0.37-1.60
Chronic or traumatic ulcer	133/448	0.84	0.49-1.44		
Dermatitis	73/448	0.86	0.44-1.68		
Diabetes (type 1 or 2)	140/448	1.34	0.81-2.22	1.34	0.76-2.35
Liver Cirrhosis	32/447	2.17	0.98-4.77	3.10	1.22-7.85
Infected foreign body[a]	45/431	0.41	0.14-1.16		
Chronic renal replacement therapy	56/448	1.12	0.55-2.24		
Glucocorticoid therapy (>5mg/day within previous month)	98/445	2.17	1.28-3.67	2.22	1.23-4.00
Immunosuppressive therapy	46/445	0.94	0.42-2.10		
Prior surgery	83/443	0.62	0.31-1.23		
Appropriate empiric antibiotic	340/422	0.60	0.34-1.06	1.33	0.53-3.33

[a]Articular prosthesis n=21, osteosynthesis material=9, pacemakers n=7, vascular prosthesis n=8

OD, odds ratio; CI, confidence interval.

Table V. Comparison of mortality within 28 days (d28 mortality) of patients with *Staphylococcus aureus* bacteremia using logistic regression. Mortality is adjusted for factors significantly associated with d28 mortality in univariate analysis.

Crude d28 mortality	% (c/n)
MSSA	15.5 % (52/336)
PRSA	17.0% (38/224)
PSSA	12.5% (14/112)
MRSA	26.8 (30/112)
d28 mortality	OR (95% CI)
MRSA vs. MSSA (all)	2.00 (1.20-3.34)
MRSA vs. PRSA	1.79 (1.04-3.09)
MRSA vs. PSSA	2.56 (1.27-5.15)
PRSA vs. PSSA	1.43 (0.74-2.77)
d28 mortality adjusted for underlying conditions	
MRSA vs. MSSA (all)	2.00 (1.16-3.45)
MRSA vs PRSA	1.81 (1.00-3.26)
MRSA vs. PSSA	2.60 (1.27-5.33)
PRSA vs. PSSA	1.56 (0.78-3.15)
d28 mortality adjusted for foci of infection	
MRSA vs. MSSA (all)	2.32 (1.33-4.01)
MRSA vs PRSA	1.99 (1.12-3.56)
MRSA vs. PSSA	3.02 (1.43-6.38)
PRSA vs. PSSA	1.66 (0.79-3.48)

MRSA, Methicillin resistant *Staphylococcus aureus*; MSSA, Methicillin susceptible *Staphylococcus aureus*; PRSA, penicillin resistant *Staphylococcus aureus*; PSSA, penicillin susceptible *Staphylococcus aureus*; OD, odds ratio; CI, confidence interval.

Appendix. A complete list of variables analysed in the study

	MRSA	%	MSSA	%	PRSA	%	PSSA	%
Underlying conditions								
Male	69/126	54,8	207	55	138/252	54,8	69/126	54,8
Age (years), median	74		73		73		73	
Articular prosthesis	21/122	17,6	60/370	16,2	38/246	15,4	22/124	17,7
Body mass index (kg/m^2), median	25,6		26,5		26,4		26,6	
Cardiac disease	71/126	56,3	204/378	54	135/252	53,6	69/126	54,8
Central venous catheter (CVK)	35/123	28,5	59/358	16,5	37/237	15,6	22/121	18,2
Chronic kidney disease	44/125	35,2	111/374	29,7	66/250	26,4	45/124	36,3
Chronic or traumatic ulcer	40/126	31,7	108/378	28,6	81/252	32,1	27/126	21,4
Chronic renal replacement therapy	13/126	10,3	49/378	13	34/252	13,5	15/126	11,9
COPD or asthma	26/126	20,6	55/378	14,6	38/252	15,1	17/126	16,1
Current smoking	31/83	37,3	63/300	21	41/206	19,9	22/94	23,4
Dermatitis	17/126	13,5	60/378	15,9	44/252	17,5	16/126	12,7
Diabetes type I or II	45/126	35,7	112/378	29,6	80/252	31,7	32/126	25,4
Glucocorticoid therapy (>=5 prednisolone/d)	38/126	30,2	74/375	19,7	44/250	17,6	30/125	24
Healthcare-associated	105/125	84	212/373	56,8	139/250	55,6	73/123	59,3
Immunosuppressive treatment	13/126	10,3	37/375	9,9	23/251	9,2	14/124	11,3
Liver cirrhosis	10/126	7,9	28/377	7,4	18/251	7,2	10/126	7,9
McCabe classification, mean	1,69		1,46		1,51		1,38	
Osteosynthesis material	12/126	9,5	15/378	4	11/252	4,4	4/126	3,2
Other invasive procedure	51/125	40,8	130/373	34,9	85/249	34,1	45/124	36,3
Pacemaker	8/126	6,3	18/378	4,8	12/252	4,8	6/126	4,8
Prior surgery	38/125	30,4	61/373	16,4	42/249	16,9	19/124	15,3
Rheumatoid disease	12/126	9,5	33/378	8,7	21/252	8,3	12/126	9,5
Vascular prosthesis	7/126	5,6	15/378	4	9/252	3,6	6/126	4,8
Focus								
CVK associated infection	35/123	28,5	17/365	4,7	37/237	15,6	22/121	18,2
Endocarditis	5/121	4,1	25/362	6,9	18/241	7,5	7/121	5,8
Infected articular prosthesis	5/122	4,1	20/359	5,6	12/239	5	8/120	6,7
Infected osteosynthesis material	5/122	4,1	4/363	1	4/240	1,7	0	0
Infected vascular prosthesis	4/122	3,3	6/363	1,7	4/240	1,7	2/123	1,6
Mediastinitis	1/124	0,8	17/365	4,7	10/243	4,1	7/122	5,7
Native arthritis	4/122	3,3	23/359		17/239	7,1	6/120	5
Osteomyelitis or spondylodiscitis	11/126	8,7	48/378	12,7	31/252	12,3	17/126	13,5
Pneumonia	24/122	19,7	57/360	15,8	34/239	14,2	23/121	19
Skin or soft tissue infection	56/122	45,9	132/360	36,7	84/239	35,1	48/121	39,7
Unknown focus	19/122	15,6	65/362	18	46/241	19,1	19/121	15,7
Treatment								
Acute renal replacement therapy	8/126	6,3	4/373	1	2/249	0,8	2/124	1,6
Echocardiogram performed	34/121	28,1	170/363	46,8	111/243	45,7	59/120	49,2
Empiric antibiotic effective	37/118	31,4	349/358	97,5	235/240	97,9	114/118	96,6

Intensive care unit treatment	15/126	11,9	51/376	13,6	38/251	15,1	13/125	10,4
Invasive ventilation	13/124	10,5	28/373	7,5	20/250	8	8/123	6,5
Lowest mean arterial pressure on day 0 (mmHg), median	78		85		85		88	
Dead on day 28	30/112	26,8	52/336	15,5	38/224	17	14/112	12,5

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ORIGINAL ARTICLE

Spa type distribution in MRSA and MSSA bacteremias and association of spa clonal complexes with the clinical characteristics of bacteremia

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Abstract

The genetic distribution of invasive methicillin-susceptible (MSSA) and resistant *S. aureus* (MRSA) strains has to be addressed in order to target infection control strategies. A large MRSA epidemic caused by a certain MRSA strain (spa type 067) broke out in 2001 in our health district. We wanted to investigate the current spa type distribution in MRSA and MSSA bacteremias and assess the potential association of spa clonal complexes (spaCC) with the clinical characteristics of *S. aureus* bacteremia. One hundred nine invasive MRSA isolates and 353 invasive MSSA isolates were spa typed and grouped into clonal complexes (spaCC). Spa type distribution was compared to that of colonizing MRSA strains. Spa type and spaCC data linked to clinical information on the course of bacteremic cases was used to search for differences in virulence between strains. Spa type distribution in MRSA is less heterogenic than in MSSA. t067 dominates both in MRSA colonisations and in invasive findings. Among MSSA, no such dominating strains were found. Of spaCCs, mortality was the highest in spaCC 067 (25.6%). SpaCC 008 was more often associated with endocarditis than other CCs (22.7 vs 5.8%, $p = 0.013$), spaCC 2133 with skin infections (68.4 vs 36.4%, $p = 0.007$), and spaCC 012 with foreign body infections (25.0 vs 9.3%, $p = 0.029$) than other clonal complexes. A single successful strain can explain the major proportion of MRSA among *S. aureus* bacteremias. Certain spaCCs showed association with certain clinical characteristics. These findings suggest that *S. aureus* strains differ in their virulence and invasiveness.

Keywords *S. aureus* · Bacteremia · MRSA · MSSA · Spa · Clonal complex

Introduction

Staphylococcus aureus (*S. aureus*) is a human commensal that has a potential to cause life-threatening invasive infections. The clinical course of invasive *S. aureus* infection may vary significantly. Several host-related factors are known to attribute to the risk of developing an invasive infection [1] and to the outcome of the invasive disease [2]. Methicillin resistance is shown to be associated with a worse outcome of *S. aureus* bacteremia (SAB) [3].

The bacterium may harbour a remarkable number of virulence factors that contribute to its pathogenicity and ability to cause invasive disease [4, 5]. Some studies have suggested that certain *S. aureus* clonal lineages harbour specific sets of virulence genes and may be related to more severe disease [4, 6–8]. However, this is not a clear-cut correlation, as there are studies which have failed to show an association between a *S. aureus* lineage and invasiveness [9] or severity of the disease [10]. Many of the studies analysing the association of genetic lineage and clinical characteristic have used the multi-locus sequence

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typing (MLST) method [7, 8, 11] for distinction of separate lineages. Also, certain invasive *S. aureus* lineages have been shown to spread more efficiently geographically suggesting higher virulence of these strains [12, 13].

In Finland, spa typing has been the primary method for methicillin-resistant *S. aureus* (MRSA) strain distinction since year 2009. Spa typing is based on sequencing of the polymorphic X region of the protein A gene (*spa*). Protein A is a surface molecule of *S. aureus*. *Spa* polymorphism may have an impact on *S. aureus* virulence because protein A binds immunoglobulins and is thus thought to dampen the opsonisation-dependent complement activation [14, 15]. Protein A is also known to bind thrombocytes providing a mechanism for bacterial cell adhesion to sites of vascular injury and thrombosis [16].

A few studies have reported an association of certain spa types or spa clonal complexes (spaCCs) with severe disease [17, 18] . There are also studies that did not find any spa type association with an increased risk of death or clinical manifestations [19].

MRSA surveillance is particularly important because it provides a tool to follow control efforts to interrupt its spread. It is currently believed that new MRSA strains may emerge repeatedly in different parts of the world by horizontal transfer of the *mec* element, the central genetic determinant for methicillin resistance, to methicillin-susceptible *S. aureus* (MSSA) strains. Therefore, it is important to understand the epidemiology of MSSA, which provides the genetic reservoir from which MRSA may emerge [20]. Because MRSA strains are younger in their evolutionary timescales, a greater genetic diversity among MSSA is observed. MRSA has a predominantly regional distribution [12], which suggests that only selected MRSA lineages are widely disseminated. However, recent studies have identified increasing levels of genetic diversity among MRSA in Europe [21–23].

The aim of this study was to compare diversity of MRSA and MSSA in blood stream infections in Pirkanmaa Health District, Finland. We wanted to investigate the dominant clones and assess their potential association with the clinical characteristics of SAB.

Methods

Data collection

The present study material is based on two sources of information: our previous SAB study [24] and the MRSA database held by Infectious Diseases Unit of Pirkanmaa Hospital District [25].

In the SAB study, all MRSA bacteremia episodes notified to the surveillance system of antibiotics and infections (SAI) in Pirkanmaa County during 2004–2013 were included, and as controls, three MSSA episodes for each MRSA bacteremia episode were also included. A case was defined as a patient with at least one blood culture positive for *S. aureus* together with clinical signs and symptoms of an infection. The controls were matched by isolation year, gender and age [24]. Of recurrent episodes, only the first episode was included in the analysis. Clinical patient data was retrospectively recovered from hospital records. Infected foreign bodies were defined as infected vascular prosthesis, osteosynthesis material, pacemakers or articular prosthesis. Endocarditis was defined using the modified Duke criteria [29]. An echocardiogram is recommended for every *S. aureus* bacteremia patient, unless the disease appears to be very mild.

The MRSA database contains information on all new MRSA findings in Pirkanmaa County since the year 2001, when a MRSA epidemic broke out in Pirkanmaa County. Only the first MRSA finding from each patient recovered either from a screening sample or a clinical sample is recorded in the database. MRSA screening on hospital admission was recommended for selected patient groups (targeted screening) in years 2001–2011. Since then, a universal screening has been recommended [25]. Due to the universal screening, a large number of MRSA carriers have been found [25, 26]. In the present study, we only focus on those MRSA cases that were recovered from screening samples.

Spa typing and clonal complexes

MRSA strains are routinely typed each time when a new MRSA carrier is recognised or when a MRSA strain is recovered from a blood culture. For this study, we retrospectively spa typed all bacteremic MSSA control strains from our previous study [24] recovered in Pirkanmaa during years 2004–2013 and also those MRSA strains that had not been spa typed before year 2009.

Spa types were grouped into spa clonal complexes (spaCC) using the Based Upon Repeat Pattern (BURP) algorithm of the Ridom StaphType (version 2.2.1) software (Ridom GmbH, Würzburg, Germany), which supports the identification of epidemiologically related strains [27, 28]. Spa types shorter than five repeats were excluded, and spa types were clustered into the same spaCC if costs distance were less than 6. We used these default settings of the algorithm to create bigger groups and to increase the statistical power in the analysis of association of spaCC and clinical characteristics.

A SPSS package (version 22) was used for statistical analyses. A Fisher's exact test was used for analysis of categorical data. p value < 0.05 was considered as statistically significant.

Results

In total, 462 SAB episodes (109 of which were caused by MRSA and 353 of MSSA, respectively) covering years 2004

to 2013 were studied. Of MRSA patients, 58/109 (53.2%) were male, and of MSSA patients 192/353 (54.5%) were male. The mean age of MRSA patients was 71 years (range 28–93) and that of MSSA patients 70 years (range 23–96). Three episodes with non-typeable strains were excluded.

Distribution of the most common ($n \geq 5$) spa types in bacteremic MRSA and MSSA strains is presented in Table 1. During the study period, 14 different spa types were found among the MRSA bacteremias. The most common spa type in MRSA bacteremias was t067, which constituted 82.6% of cases (90/109). There were 10 singletons. Simpson's Diversity Index was 0.32.

In MSSA bacteremias, 154 different spa types were found. The most common spa types were t172 ($n = 28/353$, 7.9%), t084 (21/353, 5.9%) and t015 (20/353, 5.7%). There were 113 singletons. Simpson's Diversity Index was 0.98.

Four spa types (t067, t172, t015 and t008) were found both in MRSA and in MSSA bacteremias while all the other spa types were found only either in MRSA or in MSSA bacteremias (Table 1).

From year 2009 to 2013, 1645 positive MRSA screening samples from non-infected carriers were detected and 115 different spa types were found. For 14 carriers, the spa type result was not available. Ranking of the most common spa types ($n \geq 10$) in the screening samples is shown in Table 2. Distribution of these spa types in MRSA bacteremias ($n = 58$) during the same time period is also shown. Four of the most common spa types found in the screening samples were also found in MRSA bacteremias.

Table 3 presents the assignment of each spa type in spa clonal complexes (spaCCs) and their occurrence in MRSA and MSSA bacteremias. None of the spaCCs included only MRSA strains, but there were five spaCCs including only MSSA strains, namely spaCC 084, spaCC 164, spaCC 012, spaCC 2133 and spaCC 081. SpaCC could not be defined for 20 spa types with fewer than five repeats, four spa types were considered non-founders and 10 were singletons.

Analysis on the association of spaCC and the clinical characteristics of MSSA bacteremia is shown in Table 4. SpaCC 005 was excluded from the analysis as there were only two cases. SpaCC 012 was more commonly associated with foreign body infection (6/24 (25.0%) vs 24/259 (9.3%), $p = 0.029$), spaCC 008 with endocarditis (5/22 (22.7%) vs 15/260 (5.8%), $p = 0.013$) and spaCC 2133 with SSTIs (13/19 (68.4%), vs 95/263 (36.4%), $p = 0.007$) than other spaCCs. Highest D28 mortality was associated with spaCC 008, but

Table 2 Spa type ranking of the most common spa types ($n \geq 10$) in positive MRSA screening samples ($n = 1645$) and their distribution in MRSA bacteremias ($n = 58$) during 2009–2013 in Pirkanmaa. Altogether, 115 different spa types were found in the screening samples

Spa type	Screening samples		Bacteremias	
	n	%	*N*	%
t067	1017	61.8	46	79.3
t172	105	6.4	2	3.4
t008	104	6.3	1	1.7
t032	50	3.0	4	6.9
t1012	50	3.0	0	0
t596	21	1.3	0	0
t026	20	1.2	0	0
t3109	18	1.1	1	1.7
t002	17	1.0	0	0
t022	13	0.8	0	0
Spa type data missing	14	0.9	0	0
Other spa types	219	13.3	4	6.9

Table 1 Spa types and their distribution in bacteremic MRSA ($n = 109$) and MSSA ($n = 353$) isolates covering years 2004–2013 in Pirkanmaa

Spa type	MRSA		MSSA		Total	
	No. of isolates	%	No. of isolates	%	No. of isolates	%
t067	90	82.6	3	0.8	93	20.1
t032	4	3.7	–	–	4	0.9
t172	3	2.8	28	7.9	31	6.7
t3109	2	1.8	–	–	2	0.4
t015	1	0.9	20	5.7	21	4.5
t008	1	0.9	11	3.1	12	2.6
t084	–	–	21	5.9	21	4.5
t026	–	–	17	4.8	17	3.7
t160	–	–	14	4.0	14	3.0
t002	–	–	11	3.1	11	2.4
t267	–	–	11	3.1	11	2.4
t021	–	–	9	2.5	9	1.9
t127	–	–	9	2.5	9	1.9
t164	–	–	7	2.0	7	1.5
t302	–	–	6	1.7	6	1.3
t012	–	–	6	1.7	6	1.3

MRSA methicillin-resistant *Staphylococcus aureus*, *MSS* methicillin-susceptible *Staphylococcus aureus*

Table 3 Spa types and their spa clonal complexes (CC) in MRSA and MSSA bacteremias in Pirkanmaa in years 2004–2013

Spa clonal complex	Spa types	MRSA *n* (%)	MSSA *n* (%)	Total *n*
spaCC 067	t067, t002, t105, t010, t088, t11462, t14809, t2532, t3109, t3134, t3148, t509, t548, t688, t9110	95 (78.5)	26 (21.5)	121
spaCC 630	t015, t302, t073, t1510, t050, t102, t230, t4488, t550, t630, t1122, t116, t14628, t14811, t14815, t14818, t2120, t2171, t2626, t295, t487, t583, t6406, t908, t065, t1012, t1402, t040, t11344, t1248, t130, t14814, t14821, t2143, t2275, t507, t6450, t715, t9628	2 (2.5)	77 (97.5)	79
spaCC 084	t084, t085, t346, t091, t491, t5387, t094, t14819, t14865, t2623, t3024, t358, t547, t803	–	44 (100.0)	44
spaCC 164	t267, t127, t164, t731, t1236. t1429, t14630, t1497, t1508, t164, t16555, t174, t189, t2094, t3004, t4158, t521, t8225	–	42 (100.0)	42
spaCC 172	t172, t9629, t976	4 (12.1)	29 (87.9)	33
spaCC 012	t021, t012, t018, t090, t14810, t14816, t238, t275, t298, t342, t382, t710	–	25 (100.0)	25
spaCC 008	t008, t024, t064, t14817, t1617, t211, t304, t574, t596, t6837, t711, t7840	1 (4.3)	22 (95.7)	23
spaCC 2133	t160, t14629, t14820, t156, t2133, t888	–	19 (100.0)	19
spaCC 081	t078, t1102, t056, t081, t087, t2078, t660	–	10 (100.0)	10
spaCC 005	t032, t005, t1955, t1891	6 (75.0)	2 (25.0)	8
Singletons	t100, t136, t148, t488, t647, t1458, t2095, t10471, t14631, t14813	–	11 (100.0)	11
Non-founders	t159, t377, t645, t14812	–	6 (100.0)	6
Excluded	t026, t519, t287, t693, t1023, t132, t14721, t1509, t282, t362, t3812, t3929, t4571,t4906, t52, t559, t5909, t650, t779, t8543	1 (2.4)	40 (97.6)	41

MRSA methicillin-resistant *Staphylococcus aureus*, *MSSA* methicillin-susceptible *Staphylococcus aureus*

the difference was not statistically significant compared to other spaCCs ($p = 0.375$). When MRSA cases were also included in the analysis, the highest mortality was associated with spaCC 067. The difference was statistically significant when compared to other spa types (31/121 (25.6%) vs 46/283 (16.3%), $p = 0.037$).

D28 mortality across the most common ($n > 10$) spa types, namely t067, t002, t015, t084, t026, t172, t267, t008 and t160, was 25/93 (26.9%), 3/11 (27.3%), 5/21 (23.8%), 3/21 (14.3%), 2/17 (11.8%), 3/31 (9.7%), 1/11 (9.1%), 1/12 (8.3%) and 1/14 (7.1%), respectively.

Because t008 is known to be associated with pneumonias and skin infections [30], we analysed these possible associations separately. Spa t008 was found in SAB with pneumonia as an infective focus only once. In bacteremias with SSTI, t008 was found three times. t008 is included in spaCC 008; pneumonia or SSTI as an infective focus was more common in bacteremias caused by several other spaCC than in spaCC 008.

Discussion

Spa type distribution in MSSA bacteremias is more heterogenic than in MRSA bacteremias, emphasizing the clonal nature of MRSA. However, among our MRSA strains

Table 4 Association of spa clonal complex (CC) and mortality within 28 days (D28) and certain foci of MSSA bacteremia in Pirkanmaa

spaCC	D28			Pneumonia			Infected foreign body			SSTI			Endocarditis			Spondylitis or osteomyelitis			Unknown focus		
	n	Total	%	*n*	Total	%	*n*	Total	%	*n*	Total	%	*n*	Total	%	*n*	Total	%	*n*	Total	%
spaCC 630	15	77	19.5	9	73	12.3	5	73	6.8	28	73	38.4	5	73	6.8	11	77	14.3	19	73	26.0
spaCC 172	2	29	6.9	6	27	22.2	4	28	14.3	11	27	40.7	1	28	3.6	4	29	13.8	5	28	17.9
spaCC 005	0	2	0.0	0	2	0.0	0	2	0.0	1	2	50.0	0	2	0.0	0	2	0.0	1	2	50.0
spaCC 164	8	42	19.0	9	41	22.0	2	41	4.9	14	41	34.1	4	41	9.8	7	42	16.7	7	41	17.1
spaCC 084	8	44	18.2	6	41	14.6	3	42	7.1	12	41	29.3	2	41	4.9	6	44	13.6	10	41	24.4
spaCC 012	4	25	16.0	6	24	25.0	6	24	25.0	10	24	41.7	0	24	0.0	3	25	12.0	2	24	8.3
spaCC 067	5	26	19.2	2	25	8.0	4	25	16.0	12	25	48.0	2	26	7.7	5	26	19.2	2	25	8.0
spaCC 008	5	22	22.7	3	22	13.6	1	22	4.5	5	22	22.7	5	22	22.7	2	22	9.1	6	22	27.3
spaCC 081	0	10	0.0	1	8	12.5	2	9	22.2	3	8	37.5	0	8	0.0	1	10	10.0	1	8	12.5
spaCC 2133	1	19	5.3	3	19	15.8	3	19	15.8	13	19	68.4	1	19	5.3	1	19	5.3	0	19	0.0

Infected foreign bodies; vascular prosthesis, osteosynthesis material, pacemakers or articular prosthesis

spaCC spa clonal complex, *D28* mortality within 28 days, *SSTI* skin and soft tissue infection, *MSSA* methicillin-susceptible *Staphylococcus aureus*

already 115 different spa types had been found in screening samples and 14 spa types in invasive samples during 2009–2013. This finding is consistent with studies reporting on increasing diversity among circulating MRSA strains [21–23].

In our study, spa type t067 dominated both in MRSA bacteremias and in colonisation samples but was rarely found in MSSA bacteremias. As colonisation with the same *S. aureus* strain often presides infection, we expect this to reflect also to the spa type distribution of colonizing MSSA strains. Thus, t067 is almost exclusively methicillin resistant in Pirkanmaa. This might indicate that the t067 clone has acquired its methicillin resistance outside of Finland, was then imported perhaps by a single source and has then spread successfully. On the contrary, spa types t172 and t008, which were common spa types in MRSA screening samples, were also ~~a~~ commonly found in MSSA bacteremias. This might indicate that these strains have spread in Finland as MSSA but have later acquired methicillin resistance. It is also possible that they have spread in Finland as MRSA, but some lineages have later lost their *mec* elements or that both the t172 and t008 lineages harbouring MRSA and MSSA strains have been imported to Finland separately. There is evidence that MRSA strains can regain their methicillin susceptibility by spontaneous excision of the *mec* element [31]. Spa type t172 is currently the most common MRSA spa type found in Finland [32], and t008 is a successful MRSA strain in the USA [33]. Spa type t008 has also been reported to cluster in southern France where it presents as both MRSA and MSSA strains [12]. Our knowledge on the Pirkanmaa MSSA spa type distribution is only based on invasive strains as we have not studied spa type distribution of uninfected MSSA carriers. This sets limitations to our understanding of the spa type situation.

Grundmann et al. studied spa type distribution in *S. aureus* invasive infections in 25 European countries in 2006–2007 and 2011 [12, 13]. In both of their studies, the most common MRSA in blood stream infections was t032 and it was found in 14.5–17.9% of cases. It was the second most common spa type in invasive MRSA strains in Pirkanmaa but there were only 4 (3.7%) cases. According to Grundmann et al., spa type t067 was the sixth (5.2%) and the fifth (4.4%) (years 2006–2007 and 2011, respectively) most common invasive MRSA finding clustering mainly in Spain and southern France. Our study demonstrates that another t067 cluster of invasive strains can be localised to Pirkanmaa since t067 accounted for 82.6% of our MRSA strains. This indicates that a single, successful strain can cause local epidemics in geographically distant regions.

In our study, spa t067 appears to have an even greater dominance among invasive strains than among colonizing strains. This can be explained by the fact that t067 has been the dominating strain in Pirkanmaa's long-term facilities and hospitals where MRSA carriers are more vulnerable to blood stream infections [25]. Another less likely explanation for the extensive predomination of t067 in bacteremias is that this strain harbours some virulence factors facilitating its spread and invasiveness. The success of spa t067 in Spain supports this interpretation [34]. On the contrary, spa t1012 has not caused any MRSA bacteremias although it is a common finding in screening samples suggesting that this strain may lack some features needed for invasion.

We have previously reported that the incidence of MRSA bacteremias in Pirkanmaa was significantly higher than in other parts of Finland from 2005 to 2014 [35]. The majority of bacteremias were caused by t067. This indicates that one single successful strain can explain the difference in the incidence of MRSA bacteremia between regions. Since 2011, campaigns to improve hospital hygiene in Pirkanmaa have influenced the number of MRSA bacteremias and have had an effect on the number of MRSA carriers [25, 35]. Reduction of t067 carriage rates is particularly evident in long-term facilities and may also explain the dynamics seen in MRSA bacteremias as the total number of MRSA bacteremias has decreased along with the decline of t067 since 2011 [25]. In addition to improved hospital hygiene, this decline may have resulted from natural strain dynamics.

To facilitate the analysis of the association of spa types and clinical characteristics of SAB, spa types were grouped into spaCCs. We found a weak statistical association between certain spaCCs and certain clinical characteristics and outcome of *S. aureus* bacteremia. Endocarditis was associated with spaCC 008, but this finding does not confirm previous studies, as they have found conflicting associations between strain type and endocarditis [10, 36, 37]. Tristan et al. reported that MLST CC 398 was relatively common in *S. aureus* strains causing endocarditis [36], and Nethercott et al. found that MLST CC12, CC20, and spa type t160 were significantly associated with endocarditis [37]. Bouchiat et al. found no difference between bacteremic strains with or without endocarditis [10]. Nienaber compared strains from bacteremias with SSTI and infective endocarditis and found MLST CC30 to associate with endocarditis [38]. In our patient population, spa types that correspond to MLST CC30 were grouped into spaCC 012 and were mostly associated with foreign body infections. In the Pirkanmaa hospital district, an echocardiogram is recommended for every patient with SAB unless they have very mild symptoms and recover very quickly. There is a possibility that some endocarditis cases may have been missed in our study, which limits our conclusions. Prevalence of these spa types in the carrier population of MSSA also influences their occurrence in endocarditis, foreign body infections and SSTIs. As we do not know the spa type distribution of MSSA in the carrier population, these results must be interpreted with caution.

The association of D28 mortality with the most common spa types was analysed, and it was highest in t002 and t067 bacteremias. The high incidence of MRSA t067 in long-term facilities may explain its association with mortality.

Interestingly, both t067 and t002 belong to the same spaCC 067. This might indicate that these related strains share virulence factors determining the disease severity.

Many well-known community-associated MRSA strains (such as USA300) belonging to spa type t008 are reported to cause severe pneumonias and SSTI infections [30]. We did not find t008 to be associated to pneumonias or SSTIs in our bacteremic patient material.

Providing evidence of the impact of microbial genetic determinants on the clinical course of SAB is challenging due to the complex and dynamic microbe-host interaction. We found some associations between the clinical characteristics of bacteremia and spaCCs. Although there is a discrepancy between our results and previous reports, we may postulate that there is a signal that certain spaCCs (008, 067, 012) may be associated with complicated and serious forms of SAB. However, each spaCC covers a large diversity of lineages carrying a variety of virulence factors, which makes it difficult to interpret these results. Although staphylococcal protein A itself impairs host defence by restraining the opsonisation-dependent activation of the complement, our results cannot prove that variation in the spa gene directly affects the disease course of SAB. It is instead likely that several virulence factors together impact the outcome and may be linked to certain strains, as other studies have demonstrated [4, 6, 7]. Methods offering higher discrimination power, such as whole genome sequencing, may in the future throw light on this matter [39]. Interestingly, antimicrobials are known to modulate *spa* expression [40] and some authors have suggested that this effect might be strain specific [41]. Thus, the antimicrobial effect may theoretically be related to spa type.

In conclusion, the spa type of the strain may modulate the disease course, but the associations we found were weak and may have only minor clinical relevance. There are several other factors, such as patient characteristics, other virulence properties, antimicrobial resistance pattern and treatment that together determine the outcome of SAB.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was not required because this was a retrospective study design based only on the review of hospital patient record data.

Ethical approval The study was approved by the Ethical Review board of the Pirkanmaa Health District (R12254).

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Original article

Trends in incidence and resistance patterns of *Staphylococcus aureus* bacteremia

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Running Head: Incidence of *S. aureus* bacteremia

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Abstract:

Background

Staphylococcus aureus bacteremia (SAB) causes a significant burden on the population. Several infection control measures have been implemented in Pirkanmaa county to combat a local epidemic with methicillin-resistant *Staphylococcus aureus* (MRSA). We aimed to study the epidemiology of SAB and antibiotic resistance of *S. aureus* and the possible influence of improving infection control on these.

Methods

Register data from 2005 to 2015 was retrospectively analysed to study the antimicrobial susceptibility, the incidence and mortality in SAB in a population-based setting.

Results

The incidence of SAB increased during the study period from 21.6 to 35.8/100,000 population. The number of both health care associated (HA) and community associated (CA) cases increased. The incidence of MSSA bacteremia increased from 19.9 to 35.2/100,000 population in Pirkanmaa in parallel to other parts of Finland. The incidence of MRSA bacteremia was 10-fold (4.5/100,000 population) higher in 2011 than in other parts of the

country, but sank to the national level (0.59/100,000 population) in 2015. The fatality rate decreased from 22% to 17%. The proportion of penicillin-susceptible Staphylococcus aureus (PSSA) increased from 23.9% in 2008 to 43.1% in 2015.

Conclusion

The incidence of both HA and CA SAB has increased since 2005. Conversely, the proportion of MRSA and PRSA bacteremia has decreased. Promotion of infection control measures may have reduced the incidence of MRSA bacteremia but not the overall incidence of SAB. The rising proportion of PSSA enables the use of targeted, narrow spectrum antimicrobials.

Introduction

S. aureus bacteremias (SAB) are often health care associated (HA) and thus potentially preventable [1]. Surveillance is essential to guide infection control efforts, and to assess their efficacy as well as to determine the burden of SAB in health care facilities. Active surveillance and feedback reduce the rate of HA infections [2].

Some studies have suggested that the incidence of bacteremias caused by *S. aureus* has increased [3-5]. This is deemed to be due to ageing of the population, increased use of intravascular devices and emergence of methicillin-resistant *S. aureus* (MRSA) [5,6]. In 2011, Mostofsky *et al* concluded that MRSA increases the overall burden of staphylococcal diseases instead of replacing susceptible *S. aureus* strains [7]. However, a large multinational population-based study did not find an increasing incidence of SAB [8]. Recent reports have drawn attention to the surprisingly high proportion of penicillin- susceptible *S. aureus* strains

(PSSA) [9-11]. Lowy *et al* reported that the percentage of PSSA strains was less than 5% in 1998 [12]. Several laboratories have consequently discontinued testing of penicillin susceptibility [9,11]. However, recent studies found 31% penicillin susceptible strains in SAB in Sweden and 14-35% in nasal swabs in 8 European countries [9,13]. Crane *et al* reported increasing proportions of PSSA among *S. aureus* isolates (up to 15%) in the USA, where MRSA is very common [11].

A large MRSA epidemic broke out in Pirkanmaa county, Finland, in 2001. To combat the epidemic, several control measures were introduced stepwise including universal screening, isolation of MRSA patients, decolonization of selected patient groups and recruitment of infection control nurses (Table 1) [14,15]. Adherence to standard precautions in all care and use of contact precautions in the care of MRSA carriers were emphasized throughout the epidemic. We have previously shown that this led to a significant decrease in MRSA transmission in hospitals [14]. The proportion of MRSA in SAB is considered to be a valid indicator of MRSA control because, unlike MRSA in screening samples, it is probably not influenced by screening activity [16]. It is reasonable to believe that MRSA control measures may influence transmission of other *S. aureus* strains as well.

In this population-based study we studied the epidemiology and mortality of SAB focusing on both methicillin and penicillin resistance of the strains. We also investigated if improving infection control measures reduces the incidence of HA episodes.

Methods

The study was conducted in Pirkanmaa county, Finland, with 526941 inhabitants (2015). There is one tertiary care hospital (TAUH), four secondary hospitals, 20 health care centres and approximately 200 institutions for long-term care in the county. The city of Jämsä with 21542 inhabitants (2015) joined the district in 2013 and therefore cases from and the population of Jämsä were excluded from the study.

A case was defined as a patient with at least one blood culture positive for *S. aureus* associated with signs and symptoms of infection. If a single patient had repeated bacteremias, each bacteremic episode was regarded as a distinct case if the interval between episodes was three months or more. All cases of SAB diagnosed in Pirkanmaa county from 2005 to 2015 were included. Data on SAB episodes was retrieved from the National Infectious Diseases Register (NIDR) at the National Institute for Health and Welfare (THL). All blood cultures in Pirkanmaa county are examined at a central laboratory, Fimlab, and positive blood cultures are notified to NIDR. The date, identity code, age, gender and information on methicillin resistance of *S. aureus* isolates are recorded in the NIDR.

Health care association (HA) was defined according to the Finnish Hospital Infection Program (SIRO) criteria [17]. A case was defined as HA if symptoms started more than 48 hours after hospital admission or if bacteremia was related to surgery within the past 30 days or other invasive procedures within the last 10 days. Infections in patients on chronic renal replacement therapy or in patients with neutropenia due to treatment of malignancy were classified as HA.

Classification of HA or community association (CA) was based on three methods. 457 cases were HA-SAB in TAUH and were included in the surveillance data of the SIRO. 1020 cases were classified by review of individual patient charts by the investigator (EJ). For the rest of

the cases (n=50), neither SIRO data nor hospital records were available and these cases were classified using the national hospital discharge database.

The number of cases was analysed in relation to the size of the population and in relation to the number of blood cultures taken each year. The incidence of SAB in Pirkanmaa was compared to the incidence in other parts of the country. Mortality was defined as death within 28 or 90 days. Data on outcome and penicillinase production of isolated strains was acquired from hospital and laboratory databases.

Two blood culturing instruments were used during the study period, until 2007 BACTEC 9240 (BD Diagnostic Systems, Sparks, MD, USA) and from 2007 BacT/ALERT 3D (bioMerieux SA, Marcy L'Etoile, France). Two sets (one bottle for aerobes and one for anaerobes) were taken at each sampling. Susceptibility testing was performed at Fimlab using the Clinical and Laboratory Standards Institute (CLSI) guideline until 2011 and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines thereafter [15]. The presence of the *mec*A gene was tested at THL using GenoTypeVR MRSA (HainLifescience). Penicillinase production was detected using disk diffusion test and cloverleaf test.

The study was approved by the Ethical Review board of the Pirkanmaa Health District. Permission to use the national registers was granted based on this approval, as they are public data (https://www.thl.fi/ttr/gen/rpt/tilastot.html). SPSS (Version 22.0, IBM Corp., Armonk, NY, USA) was used for statistical analysis.
Categorical data was analysed by the X^2 test.

Results

The total number of SAB cases in Pirkanmaa notified to NIDR during the eleven year study period was 1527 in 1423 patients. The median age of cases was 67 years (range 0-100) and 61.8% (944/1527) were male. Of cases, 54.7% (835/1527) were HA (Table 1).

The annual incidence of SAB in Pirkanmaa ranged from 21,6 (2005) to 37.2 (2014) per 100,000 population. The overall incidence was highest in patients ≥75 years of age (117.4/100,000 population), in patients 65-74 years of age (61.4/100,000 population) and in those under 1 year of age (39.4/100,000 population). The lowest incidence was seen in patients 15-24 years of age, 6.0/100,000 population. The incidence was 35.7 per 100,000 in males and 21.2 per 100,000 in females.

The number of blood cultures taken increased from 25 801 in 2005 to 46 785 in 2015. The rate of *S. aureus* isolates per 10,000 blood cultures was 39.1 in 2005 and 38.7 in 2015.

Figure 1 presents the incidence of MRSA and methicillin-susceptible *S. aureus* (MSSA) cases notified to NIDR from Pirkanmaa County and from other parts of the country. The incidence of MSSA cases increased during the study period from 19.9 to 35.2 per 100,000 population in Pirkanmaa and from 18.8 to 37.1 per 100,000 population in other parts of the country. The incidence of MRSA cases increased steeply in Pirkanmaa until 2011 when the incidence was 4.5 per 100,000 population and then decreased to the average level of the country. In other parts of the country a slight increase was seen in the incidence of MRSA bacteremia cases.

Figure 2 presents the incidence of PSSA, PRSA and MRSA in CA and HA cases in 2005-2015 (cases/100,000 population) in Pirkanmaa county. Annual variance was observed in the

incidence of both CA and HA cases but there appeared to be an increasing trend in both groups. The incidence of CA cases was lowest in 2008 (7.9/100,000 population) and highest in 2014 (18.5/100,000 population). The incidence of HA cases was lowest in 2005 (11.5/100,000 population) and highest in 2015 (20.8/100,000 population)

During the whole study period, the proportions of MRSA, PRSA and PSSA in HA bacteremias were 12.5 % (104/835), 56.2% (469/835) and 31.4% (262/835), and in CA bacteremias 3.8% (26/692), 65.8% (455/692) and 30.5% (211/692), respectively. Most infections were caused by PRSA 60.5% (924/1527). MRSA caused 8.5% (130/1527) and PSSA 31,0 % (473/1527) of the cases. The proportion of PSSA increased from the lowest 23.9% (26/109) in 2008 to 43.1% (78/181) in 2015. The proportion of PRSA declined from 67.5% (77/114) in 2006 to 55.2% (100/181) in 2015. The proportion of MRSA was highest in the year 2008 14.7 % (16/109) and lowest in the year 2015 1,7% (3/181).

Figure 3 shows day 28 mortality per 100,000 in years 2005-2015 in PSSA, PRSA and MRSA bacteremias. The overall case fatality rate was 21,8% in 2005, 20,2% in 2006 and has since stabilized at a level of 13.9%-17.1%.

Table 2 presents demographics, setting of bacteremia and outcome in MRSA, PRSA and PSSA bacteremias. Cases with MRSA were older and more often HA. Day 28 as well as day 90 mortality was higher among MRSA cases than PRSA or PSSA cases. PSSA and PRSA did not differ significantly in their association to HA, day 28 or day 90 mortality. Day 28 and day 90 mortality differed significantly between infections with MRSA and MSSA (25,4% vs 15,5%, p=0.004 and 40.0% vs 22.8 %, p<0.001, respectively).

Discussion:

An increase in the overall incidence of *S. aureus* blood stream infections from 11 per 100,000 population in 1995 to 17 per 100,000 population in 2001 in Finland has been previously reported [5]. In a multinational study, gender and age adjusted SAB incidence in Finland rose from 19.0/100.000 population in 2000-2004 to 21.8/100.000 population in 2005-2008 [8]. Our results indicate that the burden of SAB is rising and due to increase in both community and health care settings. Regardless of rising incidence, the day 28 mortality per 100,000 population has increased only slightly and case fatality rate has actually decreased suggesting improved quality of care of SAB patients.

The incidence of MRSA bacteremia in Pirkanmaa increased significantly reaching its peak in 2011. Since then, the incidence has decreased each year and in 2015 it was slightly lower than in other parts of Finland. Both HA and CA MRSA bacteremias have decreased. This coincides with a decline of MRSA findings from screening samples in Pirkanmaa hospitals, as we have reported earlier [14]. Several interventions have been implemented over the years and are probably the reason for the decrease in the incidence of MRSA bacteremia. Our study, however, indicates that interventions launched in 2010, such as short-term infection control promotion projects and targeted screening, were not effective enough. Since 2010, three mobile infection control nurses have been working in long-term care institutions and health centers to continuously promote and supervise adherence to control practices. Also universal screening was launched in 2011. We think that these combined and sustained interventions have led to a decreased transmission of MRSA. Eventually this has led to a reduction of MRSA bacteremias as the number of people at risk of such bacteremia has decreased.

We hypothesized that the improved level of hospital hygiene would lead to a concurrent decrease in HA-MSSA bacteremias as was observed in Australia, where a 9,4% reduction in the annual incidence of hospital-onset SAB was reported [18]. However, we found increases in both CA and HA blood stream infections due to MSSA. During the same period, MSSA bacteremias increased also in other parts of Finland. NIDR does not give information on the origin of MSSA or MRSA bacteremias. Thus we do not know if HA-MSSA bacteremias have increased in other parts of Finland.

There can be several explanations for the increasing incidence of MSSA bacteremias. The overall incidence and the incidence of HA bacteremia is highest in the oldest age groups. The population ≥75 years rose during the study period in Pirkanmaa from 36 251 to 44 326. The number of invasive procedures and use of intravascular devices have probably increased. The number of blood cultures taken annually increased remarkably during the study period while the rate of isolated *S. aureus* per 10 000 blood cultures remained stabile. This indicates that in the early years of our study period SAB may have been underdiagnosed. Acute care has been vigorously centralized from primary care centers to hospitals during the study period. This may resulted in more frequent blood cultures and improved the diagnostic accuracy in severe infections. It is, however, possible that a new successful MSSA strain is spreading and causing an epidemic.

The increasing incidence of PSSA in both HA and CA cases is an important finding. Cefalosporins are commonly used in Finland, which may contribute to the relative success of PSSA strains because cefalosporins are inferior to penicillin and cloxacillin in the treatment of PSSA infections [19]. Crane *et al* [11] discussed that the reason for the increase in PSSA strains noted in New York may be a heavy reliance on vancomycin, which may have created a

niche for these strains. This is not a probable explanation of our findings. Due to the low incidence of MRSA bacteremia in Finland, vancomycin is not used in the empirical treatment of sepsis. It has not been recommended in empiric therapy even in Pirkanmaa except in patients who are known MRSA carriers in whom it is combined with a cephalosporin. PSSA might also spread in a clonal manner, as Resman et al suggested [9]. In that case, a few successful strains may explain the increasing incidence of PSSA. In the era of growing antibiotic resistance, it must be kept in mind that penicillin in the treatment of PSSA could theoretically reduce the selection pressure that favours the spread of MRSA strains [20].

The distinction between PSSA and PRSA might have clinical relevance, although no apparent difference in the clinical course or outcome has been noted so far [10,21]. In this study, day 28 and day 90 mortalities were higher in PRSA bacteremias than in PSSA bacteremias, but the difference was not statistically significant. This is in line with findings by Aldman *et al* [10]. Penicillin is the drug of choice in the treatment of PSSA. It is relatively cheap, causes less irritation of the cannulated vein and the MIC values are lower for penicillin than for cloxacillin. Plasma concentrations of penicillin are higher than those of cloxacillin with conventional doses [10]. Penicillin is less associated to *Clostridium difficile* infection than broader-spectrum antibiotics [22]. Penicillin is probably underused in the treatment of PSSA bacteremia. Chabot *et al* [20]reported that penicillin was used in only 0% - 50% of PSSA bacteremia cases during 2003-2012 in the UK.

The major strength of our study is that it is population-based and thus gives an unbiased perspective on current trends and on the burden of SAB in our region. Nevertheless, it covers a restricted geographical area and *S. aureus* susceptibility patterns may vary regionally [8]. Our findings of an increased proportion of PSSA may not be generally applicable.

Additionally, the specificity of phenotypic tests to detect penicillin susceptibility is excellent but not perfect and may thus rarely produce false susceptibility results [9]. No further testing was performed to confirm the results of the disk diffusion test and the cloverleaf test.

HA of the cases was carefully studied using combined methods. However, the definition of HA infections of CDC has been criticized for overestimating the incidence of HA infections [23]. This may cause bias in assessing the impact of infection control interventions since even with the highest level of hygiene only the rate of true HA infections can be affected. As the national definition of HA in Finland is based on CDC's former definition, this may apply also to this study. However, the same definition was used throughout the study and therefore, this does not affect the trends in HA infections observed in this study. The impact of hospital hygiene interventions was not documented at the level of the individual patient.

SAB appears to cause a continuously growing burden on the population. However, during recent years, the most common resistance patterns of *S. aureus,* namely penicillin and methicillin resistance, are declining in invasive isolates in Pirkanmaa county. This contradicts the common assumption that resistant strains are gradually replacing susceptible strains. Many laboratories have discontinued testing for penicillin susceptibility of *S. aureus*. Based on our results we encourage laboratories to restart testing of penicillin susceptibility.

Disclosure of interest

The authors report no conflicts of interest

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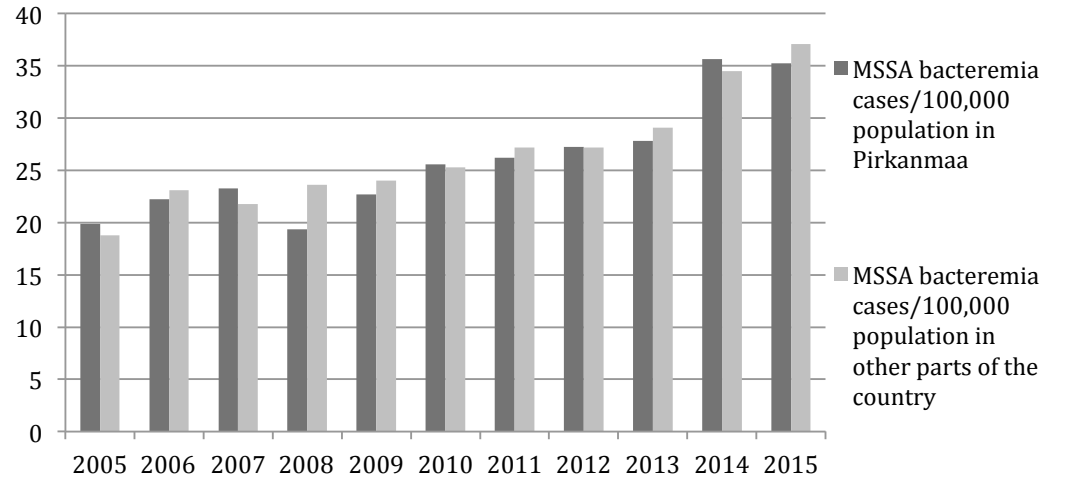
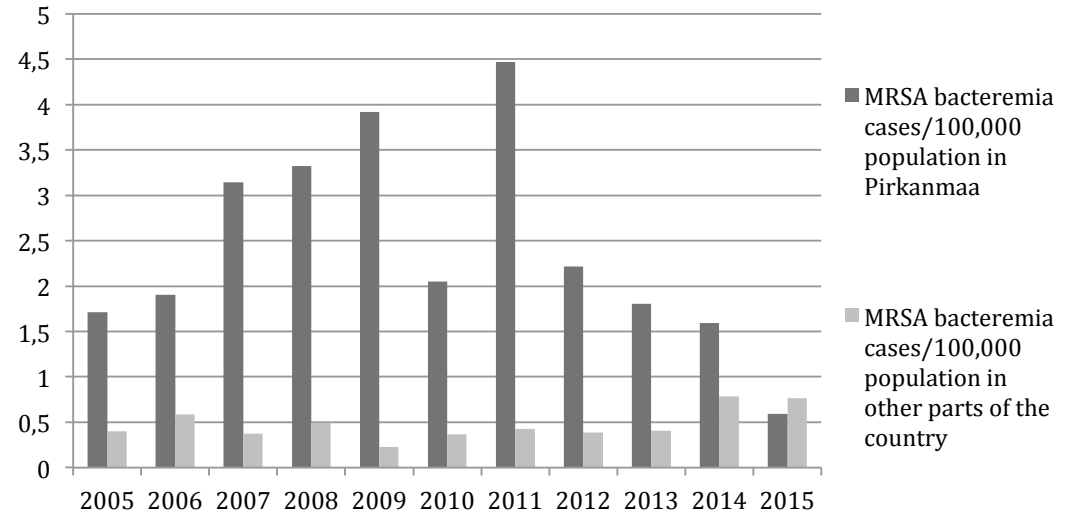
Table 1. Infection control interventions launched during the MRSA epidemic in Tampere University Hospital (TAUH), long-term facilities (LTFs) and all heath care institutions in Pirkanmaa county. Adapted from ref [14]

Type of intervention	Time
Screening of patients admitted to TAUH if they had been treated in specified institutions with known existence of MRSA	2001
Infection control promotion project in LTFs	2004
Link nurse network established in LTFs	2004-
MRSA cohort ward for surgical patients in TAUH	2008-
MRSA screening was expanded to cover all patients treated earlier in health care institutions or LTFs in Pirkanmaa county	2008
MRSA dialysis in TAUH	2009-
Infection control promotion project in TAUH	2009
Three mobile infection control nurses were recruited to visit LTFs other health care institutions regularly	2010-
MRSA screening was expanded to cover all patients admitted to health care institutions in Pirkanmaa County	2011-
Working group for MRSA epidemic was established in TAUH	2011-
Reinforcement of link nurse network in TAUH	2012-
MRSA decolonization for selected patient groups in TAUH	2013-

MRSA; methicillin-resistant *Staphylococcus aureus*

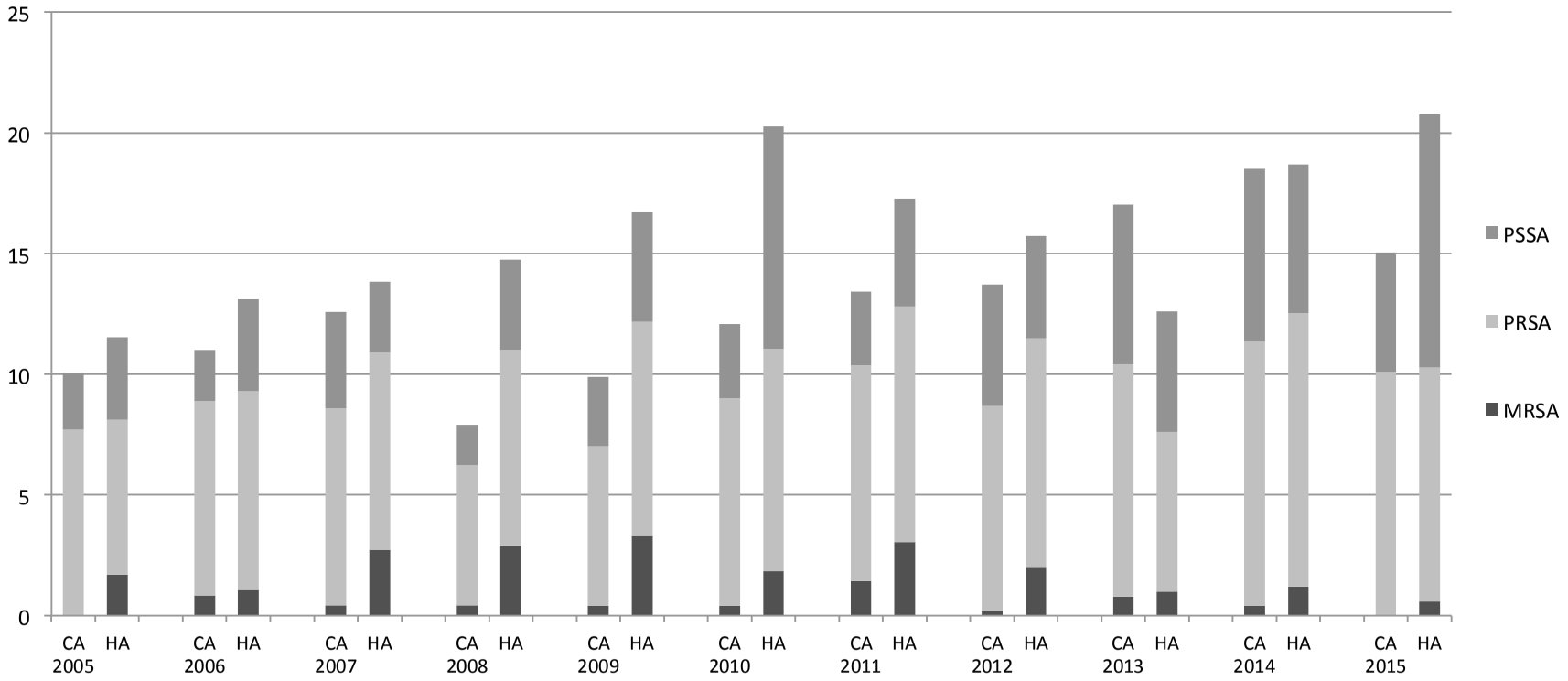
Figure 1. Incidence of MRSA and MSSA bacteremia cases in Pirkanmaa county and in other parts of Finland, 2005-2015





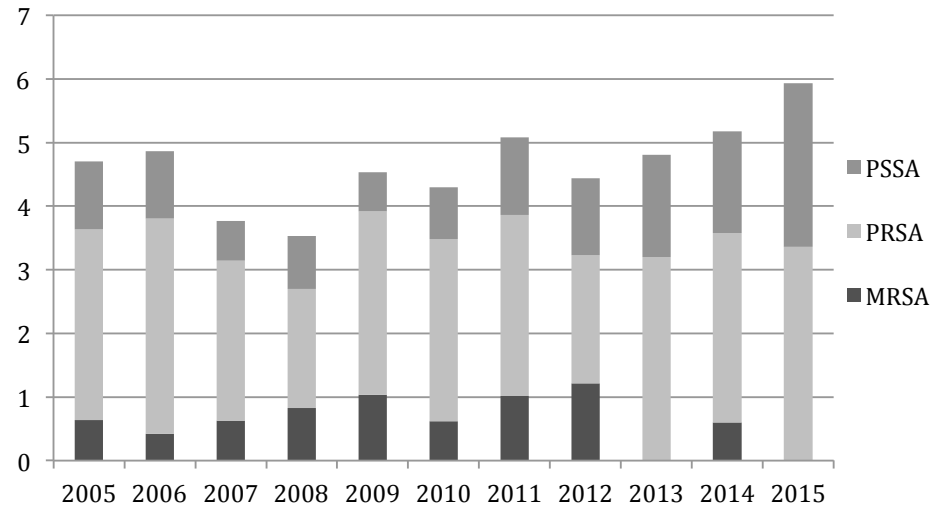
MRSA; methicillin-resistant *Staphylococcus aureus*, MSSA; methicillin-susceptible *Staphylococcus aureus*

Figure 2. Incidence per 100,000 population of PSSA, PRSA and MRSA within CA and HA bacteremia in Pirkanmaa county, 2005-2015



MRSA; methicillin-resistant *Staphylococcus aureus,* PRSA; penicillin-resistant *Staphylococcus aureus* PSSA; penicillin-susceptible *Staphylococcus aureus,* CA; community associated, HA; health care associated

Figure 3. Day 28 mortality per 100,000 population per year in PSSA, PRSA and MRSA bacteremias



PSSA; penicillin-susceptible *Staphylococcus aureus,* PRSA; penicillin-resistant *Staphylococcus aureus,* MRSA; methicillin-resistant *Staphylococcus aureus*

Table 2. Comparison of MRSA, PRSA and PSSA bacteremias

	MRSA n=130	PRSA n=924	PSSA n=473	Total n=1527	p-value
Demographics					
Male gender, n (%)	75 (57.7)	582 (63.0)	287 (60.7)	944 (61,8)	0.420
Age, median	75.5	66.0	67.0	67.0	<0.001
Origin of bacteremia					
Healthcare, n (%)	104 (80.0)	469 (50.8)	262 (55.4)	835 (54.7)	<0.001
Outcome					
28-day case fatality, n (%)	34 (26.2)	151 (16.3)	65 (13.7)	250 (16.4)	0.003
90-day case fatality, n (%)	53 (40.8)	212 (22.9)	98 (20.7)	363 (23.8)	<0.001

MRSA; methicillin-resistant *Staphylococcus aureus,* PRSA; penicillin-resistant *Staphylococcus aureus* PSSA; penicillin-susceptible *Staphylococcus aureus*